The Journal of Laboratory and Clinical Medicine

VOL. XVI ST. LOUIS, MO., SEPTEMBER, 1931 NO. 12

CLINICAL AND EXPERIMENTAL

THE INFLUENCE OF YEAST ON PROTEIN METABOLISM IN NORMAL AND DEPANCREATIZED DOGS*†

BY E. S. NASSET, PH.D., H. B. PIERCE, PH.D., AND J. R. MURLIN, PH.D.,
ROCHESTER, N. Y.

ONE of us (Pierce) has shown that the ingestion of yeast results in the retention of a larger amount of nitrogen than is obtained from the same human beings maintained on identically the same diet without yeast. There are several possible explanations of this result which have been partially considered in the paper referred to above. It was of interest to determine whether a like difference would hold for the dog and especially for the dog after extirpation of the pancreas. Meisenheimer (1919, 1921) has shown that both top and bottom yeasts contain a sufficient amount of amino acids required by the body (Völtz, 1910).

Völtz and Baudrexel (1911a, 1911b), Rubner (1916), Hawk, Smith and Holder (1919) and Murlin and Mattill (1923) have found that yeast protein is well utilized by man and animal. There is some evidence (Collip, 1923) (Euler, 1928) (Kaufman, 1928) that yeast contains an insulin-like substance besides large quantities of Vitamins B and G. MacLeod and Allan (1923), Labbe and Theodoresco (1925), von Falkenhausen (1925), Labbe, Floride, Nepveux and Hiernaux (1928), Kiech and Luck (1928), Milhorat and Chambers (1928), Kerr and Krikorian (1929), have furnished evidence that insulin affects the protein metabolism, and Hassan and Drummond (1927), have stated that a similar influence is exerted by Vitamin B complex. Moreover, the opinion has been expressed several times that the pancreatic hormone is

*From the Department of Vital Economics, University of Rochester.

Received for publication, February 10, 1931.

†This investigation was aided by a grant from the Fleishmann Company, now Standard Brands, Inc.

identical with Vitamin B (Dhar, 1926) (Randoin and Lelesz, 1925) (Chahovitch, 1925) or at least is in some way related to it. Since insulin can be absorbed in small but significant amounts from the alimentary tract of the depancreatized dog (Murlin, Sutter, Allen and Piper, 1924) (Murlin and Hawley, 1927), it seemed clearly possible that yeast given to such an animal might ameliorate the diabetic condition in two ways: (1) by reducing the intensity of the protein metabolism and (2) by improving the utilization of sugar. A study of nitrogen balance in normal dogs and of nitrogen balance and sugar excretion in depancreatized dogs therefore has been made.

EXPERIMENTAL

The dogs were fed uniform diets during the period of the experiment, the diets for normal dogs consisting of beef heart, cracker meal, lard, sodium chloride, and bone ash. After pancreatectomy, cracker meal was omitted and several of the animals were given U.S.P. pancreatin to aid digestion. From time to time yeast was superimposed on this diet.

The urine was collected over twenty-four-hour periods, the dogs being catheterized at the end of each period. A mixture of toluene and thymol was used as a preservative and several examinations made during the course of this investigation failed to reveal the presence of viable yeast or bacteria in the urine. The samples of urine were analyzed daily for nitrogen, and if the dog had been depancreatized, for glucose also.

The feces were collected and stored under 95 per cent alcohol acidified with a little sulphuric acid.* At the end of each period, the feces were marked off with charcoal and the combined daily samples were dried and pulverized, the complete sample being passed through a sixty-mesh sieve. Portions of the feces so prepared were analyzed to determine their nitrogen content.

The Kjeldahl method was used to determine nitrogen, and Benedict's quantitative sugar method was used for the estimation of glucose. The Benedict reagent was always standardized before using.

Three normal dogs were fed a uniform diet, described above, for a period of several months. The nitrogen balance of one dog (Dog I) was followed for a period of more than seven months, and of the other two (Dogs V and VI) for one month.

NITROGEN BALANCE IN NORMAL DOGS

Dog I (Normal)

The experiment on Dog I was continuous, with the exception of one month during which the dog vomited occasionally. Control periods of five to nine days each alternated with yeast periods of four to five days over most of the six months. There was one continuous control period, however, of about two and a half months, divided into periods of four to ten days in order to obtain the normal variations in nitrogen retention. The complete protocol for Dog I follows.

*To guard against the loss of NH_3 gas known to be present in larger amount in feces of human beings taking yeast than in feces from the same individuals without yeast. (Pierce.)

This was a female mongrel French bulldog weighing at the beginning of the experiment 10.5 kg. The basal diet consisted of:

		N
Beef heart	150 grams	4.61 grams
Cracker meal	40 grams	0.64 grams
Lard	10 grams	0.01 grams
NaCl	2 grams	
		5.26 grams

The experiment was started August 7, 1928
Yeast was superimposed as follows:

2 cakes Fleischmann's baker's yeast Aug. 18, 19
2 cakes dried, pulverized Fleischmann's baker's yeast Aug. 20
10 grams dried yeast cakes Aug. 20 to Sept. 11
10 grams dried starch-free yeast Sept. 11 to Oct. 22
20 grams dried starch-free yeast Oct. 22 to Nov. 11
20 grams fresh starch-free yeast Nov. 11 to Nov. 16
20 grams ordinary baker's yeast Feb. 10 to April 12

Dog I began to menstruate about February 10, 1929, and continued until the twenty-first. Following this, vomiting began and it was necessary to discontinue collections of urine in the metabolism cage. Various diets were fed until this condition was controlled. The standard diet was resumed March 22.

Discussion of Dog I.—This dog was in continuous positive balance throughout the seven months, with the exception of control period No. 9. The total amount of nitrogen retained was 149.61 gm., which would correspond to 931.7 gm. protein (N. × 6.25). Nevertheless the dog's weight during this time decreased from 10.5 kg. (Aug. 7, 1928) to 9.4 kg. (Feb. 26, 1929). The average daily retention for the entire period of 211 days was 0.71 gm. nitrogen. For the control periods totaling 150 days the average daily retention was 0.61 gm. nitrogen and for the yeast periods totaling sixty-one days it was 1.07 gm. nitrogen.* One yeast period (No. 22) of five days is not included in this average because the results on the feces were lost due to an oversight. In ten of the remaining thirteen yeast periods the nitrogen retention was definitely greater than that of the adjacent control periods.

From close comparison of the several yeast periods with the adjacent control periods it is evident that the nitrogen retained is not always due merely to the nitrogen of yeast. Sometimes the amount of extra nitrogen fed in the form of yeast is less than the extra nitrogen retained and sometimes it is more. Table I shows five comparisons of control periods with yeast periods at different stages of the long experiment. In the first comparison involving periods 2, 3, 4, 5, we have two control periods (2 and 5) one before and one following the yeast periods (3 and 4). The comparison is shown in the last two columns by the average changes noted, 0.29 gm. extra nitrogen (as yeast) ingested and 0.44 gm. extra nitrogen retained. There was a definite laxative effect in the second yeast period. The net

*Since the ammonia content of feces is increased by yeast feeding (see footnote p. 1152) it was considered possible that some of the high retention of nitrogen in this dog might be apparent only and due in reality to loss of NH_3 gas from the lower bowel. To test this possibility an attempt was made with Dog I while on yeast feeding to collect the flatus and absorb the NH_3 from it, but no gas could be obtained.

result is that one-half to two times as much nitrogen was retained (in the yeast periods as compared with the adjacent control periods) as was contained in the yeast itself.

This high retention falls in the very early stages of the experiment when, it may be supposed, the organism was not yet adjusted to the yeast regimen. What is the effect later, after the animal has been receiving yeast off and on for a period long enough to become adjusted? A satisfactory comparison is found in the eighth, ninth, and tenth periods. Each is of five days' duration and the only difference is the ingestion of 0.48 gm. yeast nitrogen in the middle period. Table I shows that the animal apparently catabolizes and excretes more and more of the extra nitrogen. Perhaps another element in the reaction is the presence of more meat nitrogen in the diet, especially in periods 18, 19, and 20 (8.94 gm. daily as compared with 5.25 gm. at the beginning).

TABLE I

COMPARISON OF NITROGEN RETENTIONS AT DIFFERENT STAGES OF YEAST FEEDING. DOG I. BEFORE PANCREATECTOMY. DAILY AVERAGES*

NO.	PERIOD DAYS	N. INTAKE GM.	N. OUTPUT GM.		N. BALANCE GM.	N. CHANGE FROM CONTROL		NET EFFECT OF YEAST EXTRA		EXTRA N. RETAINED / EXTRA N. INGESTED
			URINE	FECES		URINE GM.	FECES GM.	N. INTAKE GM.	N. RET. GM.	
2	C. 4	5.25	3.46	0.30						
3	Y. 4	5.54	3.61	0.28	C. = +1.06	−0.24	+0.13	0.29	0.44	1.52
4	Y. 4	5.60	3.66	0.59	Y. = +1.50					
5	C. 4	5.25	3.91	0.31						
8	C. 5	6.15	5.37	0.34						
9	Y. 5	6.63	5.95	0.36	C. = +0.06	+0.35	−0.14	0.48	0.26	0.54
10	C. 5	6.15	5.82	0.65	Y. = +0.32					
18	C. 5	8.94	7.62	0.46						
19	Y. 5	9.74	8.16	0.53	C. = +0.76	+0.37	+0.14	0.80	0.29	0.36
20	C. 5	8.94	7.96	0.31	Y. = +1.05					
Control period of 3 mo.										
35	C. 5	9.06	8.25	0.34						
36	Y. 5	9.58	7.72	0.58	C. = +0.29	0.59	+0.11	0.52	0.99	1.90
37	C. 6	9.06	8.36	0.59	Y. = +1.28					
Dog off experiment 4 weeks										
38	C. 5	9.06	7.38	0.27						
39	Y. 4	9.56	7.64	0.44	C. = +0.89	+0.01	−0.06	0.50	0.55	1.10
40	C. 5	9.06	7.93	0.76	Y. = +1.44					
41	Y. 5	9.56	7.69	0.48						

*C, denotes control period; Y, yeast period; N, nitrogen.

In order to get the normal fluctuations of nitrogen retention over a considerable period of time, the dog was taken off yeast entirely for nearly three months (periods 21 to 34 inclusive). There were 14 metabolism periods and in 11 of these the daily intake of nitrogen and other foods was precisely the same. The diet was as follows:

Diet Nov. 16 to Jan. 20

	Gm.	*Gm. N.*
Beef heart	275	8.44
Cracker meal	30	0.48
Lard	20	0.02
Bone ash	5	8.94
NaCl	2	

The average daily intake of nitrogen for these 11 periods was 8.94 gm. The average daily excretion was 8.43 gm., leaving an average daily retention of 0.51 gm. The variations in the daily retention run from 0.14 gm. to 0.77 gm. The extreme deviations from the mean are –0.37 gm. to +0.26 gm., i.e., from –73 per cent to +51 per cent, but the average deviation without respect to sign is 0.13 gm. or 25 per cent of the mean. This is not an excessive variability considering that it was impracticable to make daily analyses* of the beef heart, which was fed moist and fresh. The actual error involved with a total average excretion of 8.43 gm. is only 1.5 per cent.

Any change of retention due to yeast obviously must exceed this 0.13 gm. Yeast was resumed on February 10 and we have another good opportunity for comparison of the effect before the organism had become fully adjusted to yeast in periods 35, 36 and 37, where again the only difference of moment is the ingestion of 0.52 gm. yeast nitrogen in the middle period. The net effect on retention is that the addition of 0.52 gm. yeast nitrogen daily is accompanied by an *extra retention* of 0.99 gm. This duplicates the experience when yeast was first started.

Immediately following the periods just compared, the dog became sick and began vomiting a portion of the food. She was off of the experiment as a consequence for just over a month. When returned to the standard diet the retention of nitrogen during the first control period seemed to be excessive. In spite of this, a comparison of the average values of the control periods (38 and 40) with the average of the yeast periods (39 and 41) again reveals a diminution of nitrogen excretion during yeast feeding. The net effect (Table I) on retention was that the ingestion of 0.50 gm. yeast nitrogen caused an extra retention of 0.55 gm. of nitrogen daily.

An explanation of the difference in behavior early in yeast feeding as compared with a later stage, which naturally suggests itself, is that before the animal becomes adjusted the nucleoproteins, which are known to be very slowly metabolized, are retained and that this retention involves a synthesis of proteins which requires the participation of other amino acids (or metabolites) than those which come from yeast. After a period of adjustment of several weeks, however, the nucleoproteins are more and more completely broken down and excreted, perhaps because the nucleases and nucleotidases of the liver have been increased. From the retention of one and one-half times the amount of nitrogen fed the dog progressed successively to the stage where she retained one-half as much and finally only a third as much. The

*The average of 19 analyses of lean beef heart made during this investigation was 3.03 per cent nitrogen the analyses ranged between 3.22 per cent and 2.83 per cent.

dog was off yeast for nearly three months, after which, when yeast was resumed, she retained twice the amount fed, and later an amount equal to the yeast nitrogen fed.

After pancreatectomy, Dog I exhibited a marked hypersensitiveness to insulin. Suspecting that the long yeast feeding might have been the cause of this, two other dogs (Nos. V and VI) were fed a constant diet, containing yeast, for a *period of six months prior to the removal of the pancreas*, to determine any degree of hypersensitiveness to the same insulin. A study of nitrogen balance was also made, the metabolism periods beginning one month before and ending approximately six weeks after the pancreatectomy. The dogs were off diet for twelve days after the operation. The results on these dogs while normal are all that will be considered here.

PROTOCOL DOG V (NORMAL)

A mongrel female dog weighing 14.5 kg. was placed on the following diet from July 14, 1929, to January 14, 1930.

	Gm.	*N. Gm.*
Beef heart	350	10.61
Cracker meal	65	0.98
Lard	20	0.01
Yeast*	20	0.47
NaCl	4	12.07

*Fleischmann's baker's.

January 6, 1930, one month before operation, two control periods were alternated with yeast periods, a study being made of nitrogen balance.

Discussion of Dog V.—The results given in Table II show that this dog was in positive nitrogen balance and gained weight during each of the four metabolism periods. The animal showed an average nitrogen retention in the

TABLE II

DOG V (NORMAL). ON DIET 7-14, 29; RESULTS SHOWN JAN. 6 TO FEB. 2, 1930. DAILY AVERAGES

NO.	PERIOD DAYS	FECAL N. GM.	URIN. N. GM.	EXCR. GM.	N. INGEST. GM.	N. BAL. GM.	WGT. KG.
1	Y. 8	0.38	9.94	10.32	12.07	+1.75	14.7
2	C. 8	0.24	10.61	10.85	11.60	+0.75	14.9
3	Y. 7	0.27	10.36	10.63	12.07	+1.44	15.1
4	C. 7	0.35	10.19	10.54	11.60	+1.06	15.3

yeast periods of 1.60 gm. as compared with a daily retention of 0.91 gm. for the control periods. The extra retention is nearly twice the amount of nitrogen in the yeast fed. This is in marked contrast to Dog I in which an adaptation to the excretion of yeast nitrogen had apparently developed after a long period of yeast feeding. The nitrogen utilization was exceptionally good, averaging 97 per cent. The stools were small, hard and very dark in color during all of these periods. *Bowel movements occurred only at three- to four-day intervals.*

PROTOCOL DOG VI (NORMAL)

A mongrel female dog weighing 15.5 kg. was placed on the following diet from September 12, 1929, to January 14, 1930:

	Gm.	*N. Gm.*
Beef heart	400	12.12
Cracker meal	75	1.14
Lard	20	0.01
Yeast (dry)*	10	0.18
NaCl	4	
		13.45

*Northwestern.

The experiment on this dog paralleled that of Dog V, the diets being approximately the same with the exception of the yeast used. One month before pancreatectomy two control periods were alternated with yeast periods and a study of nitrogen balance was made.

Discussion of Dog VI.—The results of the study of nitrogen balance in Dog VI are given in Table III. Nitrogen retention was not increased by yeast ingestion. If the values for nitrogen balance in Table III are averaged for the two control and two yeast periods, it is seen that in both cases the retention was slightly over 0.5 gram daily. The intake of yeast nitrogen was low, amounting to 0.18 gram daily. Nitrogen utilization was excellent, averaging 97 per cent. *Bowel movements were infrequent, every three or four days, and the stools were firm and dark.* The weight of the animal increased during the month in which nitrogen balance was studied.

TABLE III

DOG VI (NORMAL). (ON DIET SEPT. 12, 1929; RESULTS SHOWN JAN. 6 TO FEB. 2, 1930.)
DAILY AVERAGES

NO.	PERIOD DAYS	FECAL N. GM.	URIN. N. GM.	N. EXCR. GM.	N. INGEST. GM.	N. BAL. GM.	WGT. KG.
1	Y. 8	0.46	11.67	12.13	13.45	+1.32	13.0
2	C. 8	0.14	12.25	12.39	13.27	+0.88	13.5
3	Y. 7	0.54	13.20	13.74	13.45	−0.29	13.5
4	C. 7	0.44	12.70	13.14	13.27	+0.13	13.6

NITROGEN BALANCE AS AFFECTED BY YEAST IN DEPANCREATIZED DOGS

Dog I (Diabetic)

The pancreas was completely removed from Dog I on July 10, 1929. For several days immediately following the operation 5 units of insulin were administered subcutaneously. The animal, however, developed insulin shock with every dose and insulin, consequently, was discontinued. Another dog (IV) of about the same weight, operated upon on the same day, received as high as 20 units of the same insulin at a single dose without showing any evidence of shock.

On July 20, Dog I was placed on the following diet:

	Gm.	*N. Gm.*	
Beef heart	380	11.59	
Lard	15	0.1	
NaCl	4		
		11.60	total N. control periods
Yeast	20	0.48	
		12.08	total N. yeast periods

The yeast used was fresh, starch-free baker's yeast. The metabolism periods were started July 20, 1929, and control and yeast periods were alternated until Aug. 13, 1929, when chemical analyses of the urine and feces were discontinued. The same mode of collecting urines and marking off the feces was followed as previous to operation.

From August 13 to September 12, 1929, this dog was kept on the control diet plus 20 gm. of fresh starch-free baker's yeast daily. After September 12 and to September 28, the beef heart was increased to 400 gm. and the yeast to 30 gm. daily. The dog was killed on September 28, 1929, having survived without insulin for ten weeks. No pancreatic tissue was visible at autopsy. Preservation of the intestine for sectioning, however, was overlooked.

The results of the nitrogen balance study and the ratio of dextrose to nitrogen in the urine are shown in Table IV.

TABLE IV

N. BALANCE IN DEPANCREATIZED DOG ON AND OFF YEAST. DOG I WAS OPERATED UPON ON JULY 10, 1929. PLACED ON DIET ON JULY 20, 1929. NO INSULIN AFTER JULY 16, 1929. TABLE BEGINS JULY 20, 1929

		AVERAGE DAILY						AV. WT. PERIOD KG.
NO.	PERIOD DAYS	FECAL N. GM.	URIN. N. GM.	URIN. GLUCOSE GM.	D:N	N. INTAKE GM.	N. BALANCE GM.	
1	C. 5	3.17	7.77	14.8	1.90	11.60	+0.66	9.6
2	Y. 5	4.20	7.16	14.0	1.95	12.07	+0.71	9.4
3	C. 5	4.22	7.50	16.4	2.19	11.60	−0.12	9.1
4	Y. 5	4.61	6.58	15.7	2.39	12.09	+0.90	8.7
5	C. 4	3.44	6.46	14.7	2.27	11.60	+1.70	8.4

Discussion of Dog I (Diabetic).—A striking difference in the daily output of fecal nitrogen in the depancreatized dog as compared with the same dog before pancreatectomy is seen at once in the first nitrogen column of Table IV. The absence of the pancreas has cut down the utilization of the protein fed from the neighborhood of 95 per cent (Table I) to approximately 67 per cent. It is, perhaps, surprising that the reduction is not greater than this. The dog was exceptional indeed in showing a positive balance after pancreatectomy in all of the periods except one (the second control) when the daily negative balance was 0.12 gm. The retention of nitrogen, however, was not much greater in the first yeast period than in the first control, and if one were judging the effect of yeast by these two periods alone, it would be negligible. It seems fairer, however, to compare the yeast period in each

case with the two adjacent control periods, as was done wherever possible with the normal dog, and on this basis the yeast has produced a plus difference of 0.44 gm. daily. This is but little less than the amount of yeast nitrogen fed (0.47 to 0.49 gm.) daily. If this result were obtained regularly, it would appear that the effect on nitrogen balance is merely that of retention of the yeast nitrogen. Attention should be drawn, however, to the diminished nitrogen output through the urine in the yeast period as compared with the two control periods. Since the depancreatized dog is dependent almost wholly on peptic digestion, at least for the early-stage digestion of proteins, one might be inclined to favor the idea that yeast has interfered with digestion. This is not borne out by subsequent experiments. Judging by output of nitrogen through the feces, the yeast produced a laxative effect.

The second comparison of a yeast period with control periods is not so clear cut. In the final control period there was diminution of both urinary and fecal nitrogen to such an extent as to produce a very great increase in the apparent retention. We believe this is not quite the correct picture, but for what reason it is distorted we do not know. Again the yeast appears to have caused a laxative effect.

This dog did not acquire a severe grade of diabetes at any time so long as the analyses were continued. The D:N ratio started at 1.90 for the first control period and at the end was only 2.27. It does not seem to have been affected either way by the yeast. *The dog survived ten weeks after the last dose of insulin was given.* It would be stimulating to believe that the yeast feeding, either before operation or after, had something to do with this very unusual record of survival without insulin, but we have no proof that this is true.

DOG II (DIABETIC)

A female mongrel dog, weighing 15.7 kg. two days after operation, was depancreatized on August 30, 1929. She was given the usual dosage (1½ clinical units per kilogram) of insulin daily until the abdominal wound was properly healed. On September 6 the animal was placed on the following diet:

	Gm.	*N. Gm.*	
Beef heart	300	9.21	
Lard	15	0.01	
NaCl	3		
Bone ash, variable			
		9.22	total N. control periods
Yeast	20	0.49	
		9.71	total N. yeast periods

The second control period was just like the first in all respects, but in the second yeast period (September 23) the beef heart was increased to 400 gm., and 15 gm. of Merck's U.S.P. pancreatin was added, making the total intake of nitrogen 11.87 gm. daily. The dog then contracted a rather severe case of cystitis and consequently had to be taken off the experiment for about ten days. One more control period of five days and a concluding yeast period of five days were run after which the experiment was discontinued because the dog had become extremely emaciated and very difficult to catheterize. The dog survived, however, until November 14, a total of forty-three days after insulin was withdrawn.

Discussion of Dog II.—Table V gives a summary of this experiment. The protein metabolism alone indicates that this dog had a much more severe diabetes than Dog I but the D:N ratio shows that the disturbance to sugar metabolism was at times less than the usual severity for this type of diabetes. The "Minkowski ratio," so called, is 2.8 D to 1 N in the urine of meat-fed dogs after pancreatectomy. This dog started off with a D:N of 2.95 and ended with 1.92, the average for the entire period of uranalysis being 2.27. The yeast appears to have had some effect on the level of the D:N ratio, for the average of the control periods is 2.53 and for the yeast periods 2.14.

TABLE V

N. BALANCE OF DEPANCREATIZED DOG ON AND OFF YEAST. DOG II WAS OPERATED UPON ON AUG. 30, 1928. PLACED ON DIET ON SEPT. 6, 1928. NO INSULIN FROM SEPT. 5 TO OCT. 18, 1928. (PERIODS 1-6 INCL.)

		AVERAGE DAILY						AV. WT. PERIOD KG.
NO.	PERIOD DAYS	FECAL N. GM.	URIN. N. GM.	URIN. GLUCOSE GM.	D:N*	N. INTAKE GM.	N. BALANCE GM.	
1	C. 5	2.35	9.98	29.5	2.95	9.22	−3.11	13.7
2	Y. 5	2.67	9.87	25.2	2.55	9.71	−2.83	13.2
3	C. 5	3.46	9.83	22.8	2.32	9.22	−4.07	12.2
4	Y. 5	3.27	11.06	21.6	1.95	11.87	−2.46	11.2
		Dog off experiment with cystitis						
5	C. 5	3.07	12.00	27.8	2.32	12.87	−2.20	9.3
6	Y. 4	3.16	11.75	22.5	1.92	13.23	−1.68	8.4
	Off experiment.							
	Killed with chloroform; 43 days without insulin.							

*Corrected for carbohydrate of pancreatin where this was fed.

The nitrogen balance certainly was influenced by the yeast feedings, for the output is obviously lower in each yeast period than in the previous control period. With one exception this is true even of the fecal nitrogen and shows that, as judged by the excretion of protein waste, *yeast does not invariably produce laxation.* The average daily nitrogen loss during the three control periods was 3.13 gm. and in the yeast periods 2.32 gm. The amount spared was about 60 per cent more than the amount of nitrogen ingested in the yeast.

Pancreatin* was first fed in the fourth period (yeast) and continued thereafter until the termination of the experiment. A comparison of the control periods on either side of this yeast period gives some information concerning the possible influence of pancreatin on nitrogen utilization (difference between intake and fecal nitrogen). Without pancreatin it was 62 per cent; with pancreatin it increased to 76 per cent. Since the D:N ratio was the same in both periods, there was no effect on sugar metabolism. The considerable lowering of the average D:N ratio in the yeast periods (from 2.53 to 2.14) is apparently a consequence of yeast feeding.

*Merck's U. S. P. pancreatin, the product used, contained carbohydrate which on hydrolysis with 2.5 per cent HCl gave 79.5 per cent of reducing substance calculated as glucose.

DOG III (DIABETIC)

A female mongrel dog of about 12.0 kg. weight before operation was depancreatized on Feb. 27, 1929, and was given insulin subcutaneously until March 11, when she was placed on the following diet:

	Gm.	*N. Gm.*
Beef heart	310	9.53
Lard	25	0.03
Pancreatin	10	0.37
NaCl	2	
		9.93 Total N. control periods.

The second control and second yeast periods were just like the first in all respects. This dog was fed pancreatin from the start, but it was increased from 10 to 20 gm. (April 7), making the total nitrogen intake 10.04 gm., and in the third yeast period three doses each of 10 gm. of yeast in suspension in water were given by stomach tube. The pancreatin was continued at 20 gm. to the end of the experiment which was terminated on April 20. The dog was killed May 2, and no trace of pancreatic tissue was to be seen macroscopically. The dog survived thirty-nine days after insulin was withdrawn.

Discussion of Dog III.—As mentioned previously, the pancreatin was found to contain a high percentage of carbohydrate, probably dextrin. When correction is made for the glucose equivalent of this carbohydrate, the D:N ratios show that this dog had a less severe grade of diabetes than Dog II (see Tables V and VI). The average for the entire experiment was 1.99; for the control periods 2.06 and for the yeast periods 1.90. There is a slight difference here in favor of the yeast periods, but the experiment so far as the influence of yeast on the carbohydrate metabolism is concerned is somewhat vitiated by the giving of pancreatin in different amounts throughout the entire experiment. The favorable effect of pancreatin on the utilization of nitrogen again appears in the last two control periods. The amount of pancreatin fed was doubled on April 7 (period 5), and continued at this level until the end of the experiment. The average nitrogen utilization for the first two control periods was 78 per cent and for the last two 85 per cent. Contrast this with 63 per cent for Dog I and 62 per cent for Dog II (before pancreatin was added) and 70 per cent for Dog IV. Digestion was improved from 10 to 15 per cent according to this showing.

TABLE VI

N. BALANCE OF DEPANCREATIZED DOG ON AND OFF YEAST. DOG III WAS OPERATED UPON FEB. 27, 1929. PLACED ON DIET MARCH 11, 1929. NO INSULIN MARCH 12 TO APRIL 20, 1929. TABLE STARTS MAR. 14, 1929

		AVERAGE DAILY						AV. WT. PERIOD KG.
NO.	PERIOD DAYS	FECAL N. GM.	URIN. N. GM.	URIN. GLUCOSE GM.	D:N*	N. INTAKE GM.	N. BALANCE GM.	
1	C. 5	2.72	8.82	19.6	2.22	9.92	−1.62	10.7
2	Y. 5	2.01	8.31	14.7	1.77	10.43	+0.11	10.4
3	C. 5	1.71	9.49	21.1	2.22	9.92	−1.29	9.9
4	Y. 5	2.06	9.58	21.0	2.19	10.43	−1.21	9.5
5	C. 6	1.49	9.26	17.2	1.85	10.04	−0.72	9.0
6	Y. 5	1.88	9.55	16.7	1.75	10.78	−0.65	8.7
7	C. 6	1.49	9.31	18.2	1.95	10.29	−0.50	8.4

*Corrected for carbohydrate of pancreatin.

Considering all of the control periods, the average daily loss of nitrogen was 1.03 gm. and in the yeast periods 0.58 gm., which is 0.08 gm. more than the extra nitrogen ingested in the form of yeast. Comparison of the average D:N ratio for the first two control periods with that of the last two control periods reveals a greater difference than was exhibited between the yeast and control periods for the whole experiment. It cannot be inferred from this that the apparently favorable effect of yeast on sugar metabolism is significant.

That the yeast favored the retention of nitrogen is clearly shown by comparison of the first yeast period with the two adjacent control periods. The average loss in these two periods was 1.45 gm. nitrogen daily, while in the yeast period there was an average daily gain of 0.11 gm., making a difference of 1.56 gm. nitrogen daily for the five days. This is more than three times the amount of nitrogen contained in the yeast itself.

DOG IV (DIABETIC)

A female mongrel dog weighing 11.0 kg. before operation was depancreatized on July 9, 1929. Insulin was given subcutaneously until July 18, when the wound was nearly healed. July 19 she was placed on the following diet:

	Gm.	*N. Gm.*
Beef heart	380	11.59
Lard	15	0.01
NaCl	4	
		11.60 Total N. control periods.

The control diet was identical throughout the experiment. In the first yeast period, 0.47 gm. of extra nitrogen was fed daily in 20 gm. of starch-free yeast and in the second yeast period, this was increased to 0.49 gm. The dog was killed August 14, 1929, twenty-six days after insulin was withdrawn.

Discussion of Dog IV.—The average D:N ratio for the whole experiment was 2.70, the average for the control periods being 2.68 and for the yeast periods 2.73 (Table VII). The nitrogen retention, or, better, the diminished nitrogen loss was appreciable when yeast was fed, only in the first yeast period. The average nitrogen loss in the two adjacent control periods was

TABLE VII

N. BALANCE OF DEPANCREATIZED DOG ON AND OFF YEAST. DOG IV OPERATED ON JULY 9, 1929. PLACED ON DIET JULY 19, 1929.* NO INSULIN AFTER JULY 18, 1929. TABLE STARTS JULY 20, 1929

		AVERAGE DAILY						AV. WT. PERIOD KG.
NO.	PERIOD DAYS	FECAL N. GM.	URIN. N. GM.	URIN. GLUCOSE GM.	D:N	N. INTAKE GM.	N. BALANCE GM.	
1	C. 5	3.20	10.33	24.3	2.35	11.60	−1.93	9.3
2	Y. 5	2.32	10.84	27.4	2.50	12.07	−1.49	8.6
3	C. 5	3.83	10.24	30.2	2.95	11.60	−2.47	7.7
4	Y. 5	5.02	9.46	28.0	2.96	12.09	−2.49	7.0
5	C. 4	3.45	10.28	28.3	2.75	11.60	−2.17	6.6

*Dog killed with ether Aug. 14, 1929; twenty-six days without insulin.

2.20 gm. daily, 0.71 gm. more than was lost during the yeast period. This is about 50 per cent more than the yeast nitrogen ingested. For the duration of the experiment the loss of nitrogen in control periods was 2.19 gm. daily and in the yeast periods 1.99 gm. Pancreatin was not included in the diet, and the losses of nitrogen in the feces were large. The average nitrogen utilization for the whole experiment was 70 per cent.

DOG V (DIABETIC)

This dog served as a normal animal before pancreatectomy and the results of the study of nitrogen balance have been presented and discussed earlier in this paper. The dog weighed 15.3 kg. before operation, and was depancreatized Feb. 10, 1930. Insulin was given subcutaneously until February 22, when the wound had healed. February 22 the dog was placed on the following diet:

	Gm.	*N. Gm.*
Beef heart	500	15.15
Lard	20	0.01
NaCl	4	
		15.16 Total N. control periods.

The control diet was the same throughout the experiment; however 20 gm. of yeast containing 0.54 gm. of nitrogen were fed in the first yeast period and 40 gm. containing 0.99 gm. nitrogen in the second yeast period. From March 1 to March 22 four metabolism periods were run to gain further information on the retention of nitrogen in diabetic dogs (Table VIII).

Discussion of Dog V.—By averaging the D:N ratios given in Table VIII, it is found that the average for the entire experiment was 2.61, the average for control periods 2.68 and for yeast periods 2.54. This slight advantage in favor of yeast is probably not significant. The average daily loss of nitrogen during the control periods was 2.67 gm. as compared with 2.27 gm. for the yeast periods. The nitrogen utilization in the diabetic animal was about 77 per cent. The amount of yeast fed was increased to 40 gm. in the last yeast period and by referring to the results in Table VIII it is seen that nearly all of the extra nitrogen was excreted in the feces. The urinary nitrogen increased only 0.07 gm. Nothing can be said about the length of life of this dog, for she was used later for other experimental work during which insulin was administered.

TABLE VIII

DOG V (DIABETIC). PANCREATECTOMY FEB. 10, 1930. ON DIET FEB. 22, 1930. NO INSULIN AFTER FEB. 21, 1930. TABLE STARTS MARCH 1, 1930

NO.	PERIOD DAYS	AVERAGE DAILY						WEIGHT KG.
		FECAL N. GM.	URIN. N. GM.	URIN. DEX. GM.	D:N	N. INGEST. GM.	N. BALANCE GM.	
1	C. 6	3.44	14.95	41.2	2.76	15.16	−3.23	12.0
2	Y. 5	3.59	14.29	36.1	2.53	15.70	−2.18	11.3
3	C. 5	3.12	14.14	36.7	2.60	15.16	−2.10	10.6
4	Y. 5*	4.28	14.21	36.2	2.55	16.14	−2.35	9.9

*40 gm. yeast.

One of the objects of this experiment was to determine the effect of a long period of yeast feeding on the sensitiveness to insulin after pancreatectomy. During the convalescent period after operation, the full dose of insulin (1½ clinical units per kilogram) was tolerated, and at no time did there appear to be any higher sensitivity to insulin.

DOG VI (DIABETIC)

This dog also served as a normal animal, a study of nitrogen balance being made prior to pancreatectomy. The results have been reported under the section dealing with normal dogs. The dog weighed 13.6 kg. before operation, and was depancreatized Feb. 10, 1930. Insulin was administered subcutaneously until February 22, when the wound had healed. February 22, the dog was placed on the following diet:

	Gm.	*N. Gm.*
Beef heart	445	13.48
Lard	30	0.01
NaCl	4	
		13.49 Total N. control periods.

The control diet remained the same during the experiment; however, 20 gm. of yeast containing 0.54 gm. of nitrogen was fed during the first yeast period and 40 gm. of yeast containing 0.99 gm. of nitrogen during the second yeast period. To obtain additional information concerning nitrogen retention in depancreatized dogs, four metabolism periods were run.

Discussion of Dog VI.—The results in Table IX show that this dog had a more severe grade of diabetes than Dog V, judging from the D:N ratios. The average D:N ratio for the whole postoperative experimental period was 2.99. The lowest ratio was obtained during the first yeast period and the highest during the last yeast period. In spite of an increased yeast intake during the final period, there was little effect on the excretion of urinary nitrogen or glucose, the final D:N ratio being but slightly higher than that of the initial period. Practically all of the extra nitrogen supplied by yeast was excreted in the feces. The daily loss of nitrogen for the control periods amounted to 0.54 gm. and for the yeast periods 0.57 gm. The average nitrogen utilization for the period of diabetes was 67 per cent.

TABLE IX

DOG VI (DIABETIC). PANCREATECTOMY FEB. 10, 1930. ON DIET FEB. 22, 1930. NO INSULIN AFTER FEB. 21, 1930. TABLE STARTS MARCH 1, 1930

NO.	PERIOD DAYS	AVERAGE DAILY						WEIGHT KG.
		FECAL N. GM.	URIN. N. GM.	URIN. DEX. GM.	D:N	N. INGEST. GM.	N. BALANCE GM.	
1	C. 6	4.23	9.98	30.1	3.02	13.49	−0.62	10.4
2	Y. 5	4.77	9.80	27.3	2.78	14.03	−0.54	10.0
3	C. 5	4.31	9.63	29.5	3.06	13.49	−0.45	9.5
4	Y. 5*	5.26	9.82	30.3	3.09	14.48	−0.60	9.2

*40 gm. yeast.

DISCUSSION OF RESULTS

The results of these experiments are summarized in Tables X and XI. In the case of the normal dogs it should be noted that the averages obtained with Dog I represent several months. Dogs V and VI were on diet several months, but the control metabolism periods were started just a month before operation. However, since the experimental routine had been thoroughly established before this, the data obtained with these animals have been included and accorded the same importance as the experiments with Dog I. Table X shows that the significant change during yeast periods was in the proportion of ingested nitrogen excreted by way of the kidneys; a diminution in the proportion of urinary nitrogen to ingested nitrogen occurring in each dog. This was quite striking in Dog I following the long uninterrupted control experiment (periods 22 to 34 inclusive). At the end of this time the animal was nearly in nitrogen balance but when more nitrogen was added to the diet in the form of yeast there was a reduction of over 10 per cent in the excretion of urinary nitrogen. The change in fecal nitrogen is probably not significant. The increase in the nitrogen retained is on the average quite considerable but in one animal there was no change.

A comparison of the *first* control period with the *first* yeast period in all of the depancreatized dogs shows that yeast feeding apparently effected the following changes:

1. In two of the six dogs the D:N was increased slightly.
   In four of the six dogs the D:N was decreased.
   The average reduction in D:N was 6.9 per cent.
2. In five of the six dogs the percentage of ingested nitrogen in the urine was decreased. In the remaining animal there was scarcely any change.
   The average reduction in urinary nitrogen was 6.5 per cent.
3. In four of the dogs the fecal nitrogen was increased.
   In two of the dogs the fecal nitrogen was decreased.
   The average reduction in fecal nitrogen was 2.6 per cent.
4. In all of the dogs the nitrogen loss was diminished.
   The average decrease in nitrogen loss was 43.8 per cent.

Comparing the averages of all of the experimental periods, the following changes were noted:

1. The D:N increased in two dogs very slightly.
   The average decrease for all dogs was 6.4 per cent.
2. The percentage of ingested nitrogen in the urine decreased in all dogs, averaging 7.4 per cent.
3. The percentage of ingested nitrogen in the feces increased in all dogs, averaging 5.2 per cent.
4. The nitrogen loss was decreased in all dogs, averaging 40.3 per cent.

From August 7 to November 11, 1928, Dog I lost 1.5 kg. in weight. This occurred in spite of a retention of about 97 gm. of nitrogen. If this had been converted to protein with the usual proportion of water, it should have been reflected in a gain in weight of 3.04 kilos.

During the continuous control period beginning November 16, 1928 (pe-

TABLE X

SUMMARY OF THE EFFECTS OF YEAST (BAKER'S) FEEDING ON NORMAL DOGS. (ALL EXPERIMENTAL PERIODS)

	PERIODS	PERCENTAGE OF INGESTED NITROGEN					
		IN URINE		IN FECES		RETAINED	
		y	e	y	e	y	e
Dog I	3, 4, 7, 9, 11, 13, 15, 17, 19, 21	84.4		5.1		+10.5	
1928	1, 2, 5, 6, 8, 10, 12, 14, 16, 18, 20		85.1		5.6		+9.3
1928-29	22 to 34 incl.*		89.7		4.1		+6.2
1929	36, 39, 41	80.4		5.2		+14.4	
	37, 38, 40		87.4		6.0		+6.6
Dog V	1 and 3	84.1		2.7		+13.2	
1930	2 and 4		89.7		2.6		+7.7
Dog VI	1 and 3	92.5		3.7		+ 3.8	
1930	2 and 4		94.0		2.2		+3.8
	Averages	84.9	89.2	4.2	4.1	+10.5	+6.7

*Continuous series of control periods with no intermittent yeast feeding.

Yeast feeding followed by $\frac{(89.2 - 84.9}{89.2} \times 100 =)$ 4.8 per cent reduction in urinary N.

Yeast feeding followed by $\frac{(4.2 - 4.1}{4.1} \times 100 =)$ 2.4 per cent increase in fecal N.

Yeast feeding followed by $\frac{(10.5 - 6.7}{6.7} \times 100 =)$ 65.7 per cent increase in N. retention.

TABLE XI

SUMMARY OF THE EFFECTS OF STARCH-FREE YEAST FEEDING ON DEPANCREATIZED DOGS

(a) Comparison of the first two experimental periods

		D:N	PER CENT CHANGE	PERCENTAGE OF INGESTED NITROGEN					
				IN URINE		IN FECES		RETAINED	
Dog I	Control	1.90		67.0		27.3		+ 5.7	
	Yeast	1.95	+ 2.6		59.3		34.8		+ 5.9
II	Control	2.95		108.2		25.5		-33.7	
	Yeast	2.55	-13.6		101.6		27.4		-29.0
III	Control	2.22		88.9		27.4		-16.3	
	Yeast	1.77	-20.3		79.7		19.3		+ 1.0
IV	Control	2.35		89.1		27.6		-16.7	
	Yeast	2.50	+ 6.4		89.8		19.2		- 9.0
V	Control	2.76		98.6		22.7		-21.3	
	Yeast	2.53	- 8.3		91.0		22.9		-13.9
VI	Control	3.02		74.0		31.4		- 5.4	
	Yeast	2.78	- 7.9		69.9		34.0		- 3.9
	Average		- 6.9	87.6	81.9	27.0	26.3	-14.6	- 8.2

(b) Comparison of averages of all of the experimental periods

Dog I	Control	2.12		62.4		31.1		+ 6.5	
	Yeast	2.17	+ 2.4		56.9		36.5		+ 6.6
II	Control	2.53		101.5		28.4		-29.9	
	Yeast	2.00	-26.5		89.8		26.1		-15.9
III	Control	2.06		91.8		18.4		+10.2	
	Yeast	1.90	- 7.8		86.7		18.8		- 5.5
IV	Control	2.68		88.6		30.1		-18.7	
	Yeast	2.73	+ 1.9		84.0		30.4		-14.4
V	Control	2.68		96.3		21.6		-17.9	
	Yeast	2.54	- 5.2		89.5		22.9		-12.4
VI	Control	3.04		72.7		31.7		- 4.4	
	Yeast	2.94	- 3.3		68.8		35.2		- 3.0
	Average		- 6.4	85.6	79.3	26.9	28.3	-12.4	- 7.4

riod 22), this animal showed a gain in weight of 0.4 kg. in about one and one-half months. This was accompanied by a retention of 28 gm. of nitrogen, the equivalent of about 175 gm. of protein.

The food intake in all animals was in most cases equivalent to 50 calories or more per kilogram.

Dog VI likewise showed a decrease in weight amounting to 0.5 kg. This loss is for the period September 12, 1929, to January 6, 1930, during which time the animal received daily 10 gm. of Northwestern dry yeast in addition to the regular diet. Withdrawal of yeast for eight days (Table III) was accompanied by a gain in weight of 0.5 kg. In the following yeast period no gain was shown, but again in the last control period there was a slight gain (0.1 kg.). That is to say, in twenty-two days she regained what she had lost in the previous long uninterrupted period of yeast ingestion.

Dog V showed 0.2 kg. gain during the long yeast period (July 14, 1929, to January 6, 1930). In the four metabolism periods preceding operation she gained 0.6 kg., exactly the same increase as was found in Dog VI in the same period.

SUMMARY AND CONCLUSIONS

1. The one invariable result of yeast feeding both in normal and diabetic dogs was the prompt decrease in the proportion of ingested nitrogen eliminated in the urine.

2. The nitrogen retention or the decreased nitrogen loss, as the case may be, runs nearly parallel to the urinary nitrogen, the few divergences being accounted for by variations in the loss of nitrogen through the feces.

3. In view of certain losses in weight, it is impossible to state whether the excess nitrogen retained with yeast feeding was built into new protein.

4. The nitrogen excretion through the feces was not essentially altered by yeast feeding.

5. On the average, yeast ingestion tends to reduce slightly the D:N ratio of depancreatized dogs.

6. There is some evidence that yeast aids in prolonging the life of diabetic dogs not receiving insulin.

REFERENCES

Chahovitch, X.: Béribéri Expérimental et Insuline, Compt. rend. Soc. Biol. **93**: 652, 1925.

Collip, J. B.: Glucokinin. A New Hormone in Plant Tissue, J. Biol. Chem. **56**: 513, 1923.

Dhar, N. R.: Der Stoffwechsel bei Diabetes und die Ähnlichkeit der Functionen innerer Sekrete mit denen der Vitamine, Chemie d. Zelle u. Gewebe **13**: 119, 1926.

Euler, Ulf von: Ein Insulinartiger Körper in der Hefe, Biochem. Ztschr. **194**: 197, 1928.

Hassan, A., and Drummond, J. C.: The Physiological Rôle of Vitamin B. IV. The Relation of Certain Dietary Factors in Yeast to Growth of Rats on Diets Rich in Proteins, Biochem. J. **21**: 653, 1927.

Hawk, P. B., Smith, C. A., and Holder, R. C.: Baker's Yeast as Food for Man, Am. J. Physiol. **48**: 199, 1919.

Kaufmann, E.: Insulinersatzmittel VIII Über antidiabetische Stoffe im Hefe und Hefenucleinsäuren, Ztschr. f. ges. exper. Med. **62**: 739, 1928.

Kerr, S. E., and Krikorian, V. H.: The Effect of Insulin on the Distribution of Non-Protein Nitrogen in the Blood, J. Biol. Chem. **81**: 421, 1929.

Kiech, V. C., and Luck, J. M.: The Effect of Insulin on Protein Metabolism, J. Biol. Chem. **78**: 257, 1928.

Labbe, Floride, Nepveux, and Hiernaux: Influence de l'insuline, sur le trouble du métabolisme azoté dans le diabéte grave, Compt. rend. Acad. Sc. **186**: 1384, 1928.

Labbe, M., and Theodoresco, B.: Action de l'insuline sur le métabolisme azoté, Compt. rend. Acad. Sc. **180**: 1438, 1925.

MacLeod, J. J. R., and Allan, F. N.: The Effect of Insulin on the Metabolism of Normal Dogs, Proc. & Trans. Roy. Soc. Canada **17**: Sect. V, 47, 1923.

Meisenheimer, J.: Die Stickstoffhaltigen Bestandteile der Hefe, Ztschr. Physiol. Chem. **104**: 229, 1919.

Meisenheimer, J.: Die Stickstoffhaltigen Bestandteile der Hefe II Mitteilung. Die Purinbasen und Diaminosäuren. Ergenbnisse, Ztschr. Physiol. Chem. **114**: 205, 1921.

Milhorat, A. T., and Chambers, W. H.: The Effect of Insulin on Protein Metabolism, J. Biol. Chem. **77**: 595, 1928.

Murlin, J. R., and Hawley, E. E.: Absorption of Insulin From the Alimentray Tract of Depancreatized Dogs When Protected by Blood Serum, Am. J. Physiol. **83**: 147, 1927.

Murlin, J. R., and Mattill, H. A.: The Laxative Action of Yeast, Am. J. Physiol. **64**: 75, 1923.

Murlin, J. R., Sutter, C. C., Allen, R. S., and Piper, H. A.: Some Favorable Effects From the Alimentary Administration of Insulin, Endocrinology **8**: 331, 1924.

Pierce, H. B.: (Not yet published.)

Randoin, L., and Lelesz, E.: Variations comparatives de la glycémie artérielle (effective et protéidique) et de la teneur du foie en glycogéne chez le pigeon soumis à un régime déséquilibre par manque de facteur hydro-soluble B, Compt. rend. Acad. Sc. **180**: 1366, 1925.

Rubner, M.: Die Resorbierbarkeit der Nährhefe, München. med. Wchnschr. **63**: 629, 1916.

Völtz, W.: The Utilization of Dried Yeast in the Animal Organism, Ztschr. Spiritusind. **33**: 588, 1910. Cited Chem. Ab. **5**: 2515, 1911.

Völtz, W., and Baudrexel, A.: Die Verwertung der Hefe im menschlichen Organismus, Biochem. Ztschr. **30**: 457, 1911a.

Völtz, W., and Baudrexel, A.: Die Verwertung der Hefe im menschlichen Organismus, Biochem. Ztschr. **31**: 355, 1911b.

von Falkenhausen, M. F.: Utersuchungen über den Eiweissstoffwechsel beim experimentellen Pankreasdiabetes, Arch. exper. Path. Pharm. **109**: 249, 1925.

THE RELATIONSHIP OF TYPE OF RADIANT ENERGY TO ITS ACTIVATION OF VITAMINS IN YEAST*

By Louis H. Jorstad, M.D., St. Louis, Mo.

IN PREVIOUS publications from this laboratory it has been noted that certain bacteria liberate vitamins into the medium about them. The Bacillus tumefaciens in its early stages of growth liberates the water-soluble Vitamin B, while in later stages apparently with death of the bacteria and stagnation of the culture, the fat-soluble Vitamin A is liberated.[1]

In our studies with x-ray it was demonstrated that smaller dosages activate Vitamin B, while larger dosages activate Vitamin A in the organism.[2] More recently we have conducted a series of experiments with activated yeast, mainly from the standpoint of Vitamin D potency. I felt, however, that with the activation of Vitamin D in a substance of this kind that other vitamins would be activated as well, principally Vitamin A or at least the so-called fat-soluble Vitamins, in that we were using as a source for our activation a mass of yeast cells in various stages or degrees of growth and dissolution.

A great amount of analytic work has been done in regard to the antirachitic vitamin placing it separate from Vitamin A. However, good results in feeding experiments have been obtained by not separating them. Clinically good results have been obtained from the therapeutic standpoint when they have been used in this way. In this series of experiments the white rat of from 50 to 80 gm. weight was used. The yeast was brewer's yeast.† In that fresh samples of the yeast were furnished twice a week, we may consider that the supply was always fresh in that it was stored in an ice box during that short interval of time. Even though the main purpose of the experiment was to determine antirachitic potency, a part of the experiment was planned to determine both antirachitic and antixerophthalmic potency. For the demonstration of the former alone, Steenbock's ration was used.[3] For the demonstration of the second, I modified a ration which we had used in the laboratory in former experiments. This ration consisted of potato starch, egg albumen, vegex, McCallum's salt mixture and crisco.[4] It was then necessary to determine the percentage of radiated yeast to add to these rations. It was found that a 10 per cent ratio gave the best results and this ratio was used throughout the whole experiment. The Steenbock's ration consisted of yellow corn, wheat gluten, calcium carbonate and sodium chloride. The food was given ad lib in dishes. Both solid bottom cages with sawdust, cleaned frequently, and screen bottom cages were used. In the con-

*From the Departments of Research and Pathology, The Barnard Free Skin and Cancer Hospital.

Received for publication, February 20, 1931.

†The unradiated yeast, the Cooper-Hewitt radiated and quartz light radiated yeast, was furnished by the laboratories of Anheuser-Busch, Inc.

trol series on my ration as outlined above all the rats developed rickets within forty days. The presence of rickets was determined by radiographs and impregnation of the bones with silver nitrate, as well as decalcification, and staining with hematoxylin and eosin. In the next setup the rats were given my ration and 10 per cent of brewer's yeast which had been exposed for ten minutes to the Cooper-Hewitt lamp. These rats developed marked rickets within sixty days. Within seventy-five days xerophthalmia was noted. In the remaining cages in this series yeast samples, each having ten minutes' longer exposure to the Cooper-Hewitt lamp than the former, were added to the same ration. The rats in the final setup received 10 per cent yeast in their ration which had been exposed to the lamp for sixty minutes. Grossly the animals in the last two cages gained in weight and showed no abnormalities during a period of ninety days. This period of time was considered ample for a study of this problem.

In a study of the radiographs, as well as the other tests, it is apparent that the basic ration induced rickets, that some antirachitic potency was present in all of the yeast samples irradiated, but that complete protective action for the period of time under observation was not present excepting in the animals receiving yeast which had been radiated for a period of fifty or sixty minutes. The animals in the last two setups did not show evidence of xerophthalmia. Definite eye changes were present in the others. The time of the appearance of this xerophthalmia varied, in progression as compared to the period of exposure to the yeast. Noting this as the earliest time it appeared in the animals on the different rations, we may say that it appeared with the ten-minute exposure within forty days, and twenty-minute exposure within fifty days, and the thirty-minute exposure within seventy-five days, and the forty-minute exposure within ninety days. This experiment was repeated and the results obtained in the first experiment were confirmed. At the sixty-day period especially, the progressive degree of calcification in the various cages is definitely made out. From the radiographic standpoint, as well as the progress in weight of the animals, the yeast radiated for fifty minutes gives results superior to those obtained with the yeast radiated for one hour.

From this first series of experiments it was definitely decided that observation of weight and appearance of the animal was not a satisfactory guide in determining the presence of rickets, at least from the standpoint of gaining any idea of the relative involvement. In the second series the Steenbock ration being used, the same procedure was followed. The gross observations showed a definite lack of gain in weight on the deficiency control ration after forty days; however, the animals showed no signs of xerophthalmia at the end of the sixty-day period. Radiographs from these rats showed the presence of rickets at the end of thirty days. The decalcification was well marked at the end of sixty days. As noted by x-ray this decalcification is especially striking in the metaphysis of the long bones. At later stages in addition to a diffuse decalcification there was marked irregularities of opacity as can be noted in Fig. 1. With the silver nitrate studies the decalcification of the metaphysis is not so strikingly revealed, but in this test we note the difference in deposition of the silver nitrate in the epiphyseal zone. The difference in

the rachitic bone as compared with the normal bone is very well marked. Fig. 2, a radiograph taken from normal bone, is included for comparative study.

The antirachitic potency of the radiated yeast samples in this setup varied in the same ratio as it did in the preceding setup. It may be more clearly demonstrated, however, that the yeast radiated for fifty minutes is more

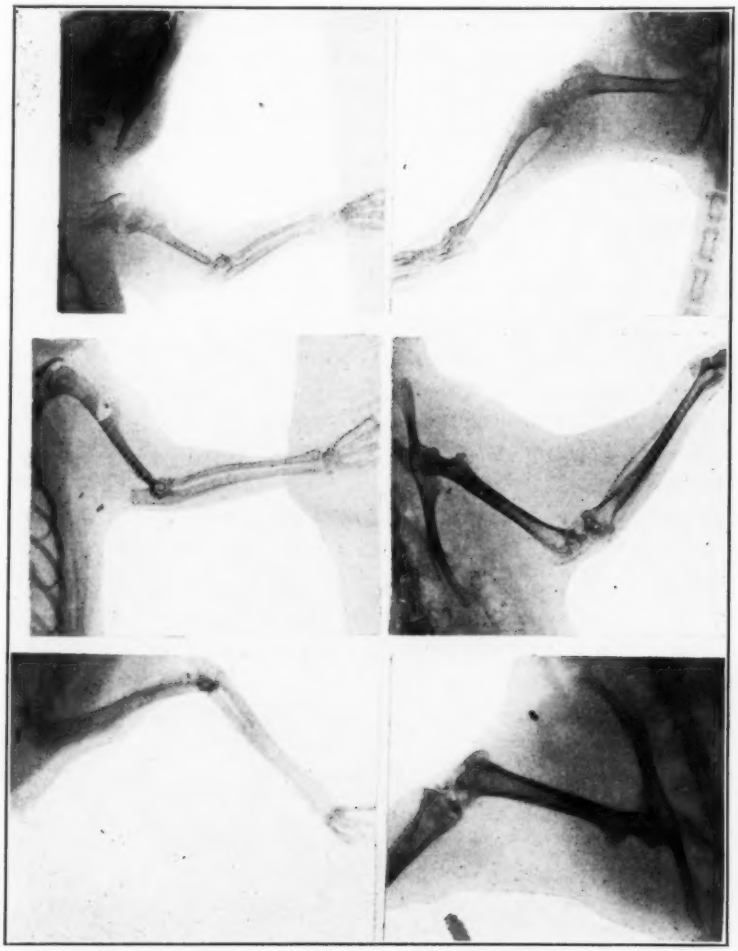


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 1.—Radiograph of rat with rickets showing decalcification anterior and posterior extremity. Steenbock ration thirty days.

Fig. 2.—Radiograph of rat on balanced ration. Note clear-cut lines at femurohumeral and radio- ulno-carpal joints.

Fig. 3.—Radiograph of rat on Steenbock rickets-inducing ration for thirty days following which 10 per cent yeast exposed to 20 ma. min. x-rays was added for a period of sixty days.

satisfactory than the yeast radiated for sixty minutes. We have at this point apparently reached the maximum of activation with the technic used. Some of the samples radiated for fifty minutes showed a definite charring, the color of the yeast being a greyish-brown instead of the definite whitish-grey color of unradiated yeast, or the samples radiated for a shorter interval of time.

The yeast radiated for sixty minutes has always a definite brownish color, and this charring may be destructive or it may change the taste of the yeast so that it is unpalatable to the animals; however, no gross differences in food consumption were noted during the ninety-day period.

We appreciated quite early in the experiment that the gradations of opacity as depicted in the radiograph may be obscured in the living animal by the enveloping musculature, and the coursing of blood through the vessels contained therein. Another factor is that of varying distance in spreading apart the limbs of the rat so as to obtain a view of the whole extremity. The distance is not the same at each point of the limb. For this reason during the course of the experiment at definite intervals, one rat from each cage was anesthetized, autopsied, extremities amputated and all the soft tissue denuded. A radiograph was taken of the denuded bone and this bone used for silver nitrate impregnation or paraffin section. This second setup verified the observations in the first that the degree of calcification varied in proportion to the period of radiation of the yeast and that the maximum of antirachitic potency was obtained with the fifty-minute exposure. In another series using the same age and weight of laboratory animal and the Steenbock ration, we determined the antirachitic potency of yeast radiated by the Steenbock method. Instead of using a Cooper-Hewitt lamp for the radiation, according to the method of Pucini, as cited above, the quartz lamp was used and the period of exposure varied from three to eleven minutes. The weight curves of these animals during a period of thirty days' observation showed nothing of interest. The radiographs at this period showed no definite decalcification in any of the animals.

From the forty-five-day to sixty-day period, however, there is a definite radiographic evidence of rickets in the rats given yeast radiated nine and eleven minutes by this method. The decalcification is more marked in the latter of the two setups. Thus we have attained our maximum activation at seven minutes with this particular technic. The weight curves and gross appearance of the animals at the ninety-day period are compatible with the radiographic data.

It has been noted in previous experiments that with the radiation of an area of skin on the white rat with 20 to 30 ma. min. of x-ray through an aperture 1 cm. in diameter, the animal will be protected against xerophthalmia for a period of thirty to fifty days longer than a rat on the same ration, but not receiving the x-ray. This amount of x-ray in this setup does not at all times induce a burn which may be discerned grossly, but microscopic changes are induced in the area radiated. Some of the gross changes are that of a transient keratosis of the skin as well as a denudation; the denudation is not complete and it is not permanent, the whole extent of the hair follicle not being destroyed.[5] This finding was confirmed by paraffin section. Taking this into consideration and keeping in mind the difference in antirachitic potency of yeast subjected to the procedures noted above, it was thought of interest to radiate samples of yeast with the roentgen ray, using 20, 25 and 30 ma. min. exposures respectively.[6] It had been noted also that a relatively

large amount of x-rays given in the form of a general body dose induced a certain degree of vitamin deficiency.[7] This deficiency was prevented by giving a dietary rich in Vitamin A. Transmutation experiments reported by several authors using different forms of radiation energy have been interesting when viewed from the standpoint of some of the findings in our experiments. There have been cases cited clinically wherein radiation has been used as a means of biologic stimulus; probably the most recent observations of this have been the satisfactory therapeutic results obtained in the treatment of agranulocytic angina with very small doses of x-ray.

From these observations it seems plausible to assume that the x-ray plays an important rôle in the activation or utilization of Vitamin A in the body and might be a source of activation of Vitamin A in other forms in nature. It has been reported in former studies from this laboratory that small amounts of x-ray given in the form of general body doses over a period of time protect the animal against a Vitamin B deficiency.[7]

We exposed the brewer's yeast to different amounts of x-ray after cutting the pound blocks into sheets one centimeter in thickness. The x-ray setup used was 10-inch distance, 9-inch spark, 90 kilovolts, 4 minutes' exposure giving 20 ma. min., etc. With this yeast and x-ray setup the greatest protection was afforded against rickets by that given the 20 ma. min. exposure, less protection being obtained with the higher exposures, No signs of Vitamin A deficiency was noted in any of the animals during the course of the experiment.

The interesting factor in these experiments is there seems to be a point wherein activation of the rickets preventing substance reaches its maximum and then becomes less, the point at which the maximum is obtained being different with the different lights used. From this one can assume that the question or factor involved in this process of activation is that of energy and in this particular series of experiments the Cooper-Hewitt lamp is the least efficient as a source when compared to the quartz light and the low voltage x-ray, the latter being the most efficient from the standpoint of time of exposure necessary (Fig. 3).

CONCLUSIONS

1. The Cooper-Hewitt lamp, quartz light and low voltage x-ray cause an activation of Vitamin D and Vitamin A in brewer's yeast fed to rats along with a dietary deficient in these vitamins. The necessary period of exposure to the light in each case varies with the type of light used. A maximum point of activation is found with each ray.

2. These findings tend to refute the idea that certain lights are specific in the liberation of certain products, such as Vitamin A by the quartz light, etc.

3. These experiments tend to establish that Vitamin D is closely allied with Vitamin A and that the liberation of these vitamins from brewer's yeast depends upon the amount of "ray" energy given.

REFERENCES

1. Burrows, M. T., and Jorstad, L. H.: On the Source of Vitamin B in Nature, Am. J. Physiol. **77**: 24, 1926.
2. Burrows, M. T., Jorstad, L. H., and Ernst, E. C.: Cancer, Vitamin Imbalance, and Roentgen-Ray Activity, J. A. M. A. **87**: 86, 1926.
3. Steenbock, H., and Block, A.: Fat-Soluble Vitamins, J. Biol. Chem. **64**: 263, 1925.
4. Jorstad, L. H.: The Relation of the Vitamins to the Reaction Induced by Coal Tar in the Tissues of Animals, J. Exper. Med. **42**: 221, 1925.
5. Jorstad, L. H., and Lane, C. W.: Gross and Microscopic Changes in the Skin Produced by X-rays and by Lipoid Solvents, Arch. Dermat. & Syph. **19**: 954, 1929.
6. Jorstad, L. H.: Some Observations on Vitamins A and D Activation of Yeast by X-ray and Other Radiation, Proc. Soc. Exper. Biol. & Med. **27**: 930, 1930.
7. Burrows, M. T., Jorstad, L. H., and Ernst, E. C.: The Effect of X-rays on the Vitamin Needs of the Organism and Cancer, Radiology **7**: 279, 1926.

NOTES ON BACILLUS WELCHII*

By A. S. Schlingman, D.V.M., M.S., Detroit, Mich.

BACILLUS welchii is probably more widely distributed in nature than any of the pathogenic anaerobes. Since it is a common inhabitant of the intestinal tracts of both men and animals, it follows that it is also found in soil, particularly that which has been manured extensively.

During the World War, a great part of which was fought over land which had been cultivated and fertilized for centuries, 0.5 to 3.5 per cent of those wounded developed gas gangrene. Since the mortality of these cases varied from 10 to 50 per cent,[1] depending on how early treatment was given, the bacteriology of the condition was the subject of extensive investigation. After the Armistice was signed, interest in gas gangrene waned somewhat only to be renewed during the last few years due to the fact that an increasing number of cases have been, and are being, met in civil practice. King[2] in a recent review of the literature and in an observance of a series of cases concludes that gas-bacillus infection occurs in civil practice as an important mortality factor in a variety of clinical conditions, including compound fractures, puerperal infection, intestinal obstruction and peritonitis with ileus, gangrenous appendicitis and pelvic or abdominal contusions. The bacteriologic data of these cases pointed to B. welchii as the predominating causative organism.

The researches stimulated by the large number of cases of gas gangrene occurring during the World War showed that B. Welchii alone or associated with other anaerobes, particularly Vibrion septique and B. edematiens, was responsible for the majority of these cases. Other anaerobes, including B. sporogenes, B. histolyticus, B. tertius, B. fallax, B. putrificus, and B. bifermentans, were also found but several of these species appear to play what may be termed preparatory rôles in that they may aid in the spread of infection by producing gas or putrid conditions, or both, in the wounds.

B. Welchii is cultivated without difficulty under anaerobic conditions or at times under partial tension. It grows readily in practically all media, especially those containing carbohydrates. Its proteolytic powers are extremely limited. Simonds[3] classifies the organism into four groups, based on the ability or inability of strains to ferment inulin and glycerin, or neither or both. However, all are alike in their toxin production. Bull and Pritchett[4] found that twenty-two strains were alike in toxin production and that each toxin was neutralized by antitoxin produced with one of the toxins selected for experiments reported in a previous publication.[5]

In Hibler medium,[6] a combination of beef liver bouillon and beef brains, growth is extremely active, the maximum being reached in from eight to

*From the Research and Biological Laboratories, Parke, Davis and Company.
Received for publication, March 10, 1931.

twenty-four hours at 37° C. Considerable gas is evolved, the liquid becomes slightly turbid and an odor of butyric acid is present. In older cultures there may be a slight softening of the brain tissue but no digestion takes place. Considerable acid is produced during the growth of the organism, the reaction of the cultures ranging from P_H 6.0 to 6.4, although the initial reaction of the medium may have been P_H 7.6 or even more alkaline.

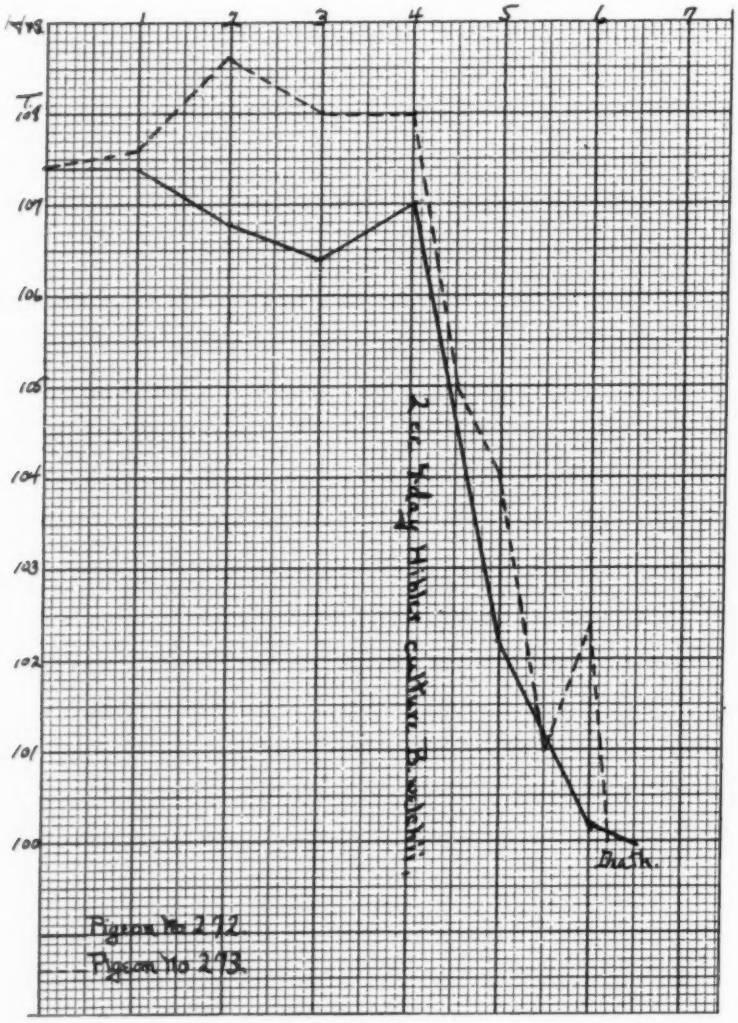


Chart 1.—Temperatures of pigeons prior to and after injection of B. welchii.

In shake cultures in a 2 per cent agar medium containing 2 per cent glucose, much gas is usually evolved, frequently being sufficient to shatter the agar. The colonies are white and essentially lenticular, and, if the seeding has been heavy, are small. When the seeding has been light the colonies are larger and very often appear broken along one edge, taking on an appearance somewhat similar to an ocarina or "musical sweet potato."

Pigeons are probably the most susceptible of any of the experimental animals to the action of B. welchii and its toxin. Intramuscular injection of pure cultures may be followed by death in from two to twenty-four hours, depending somewhat on the size of the dose, virulence, etc. Within one-half to one hour after injection of 1.5 to 2 c.c. of a twenty-four-hour Hibler culture, of such virulence that death results in three to four hours, the pigeon stands listlessly with feathers ruffled. Later the wings are held extended from the body and respiration becomes rapid. As the infectious processes advance, respiration becomes oral, the eyes become dull and finally the bird falls over dead without showing any convulsions. The temperature during

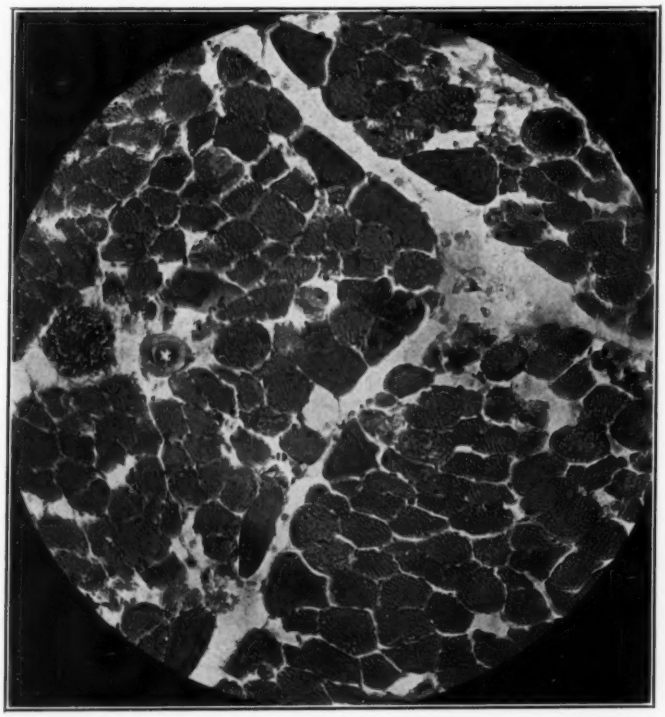


Fig. 1.—Pigeon muscle, B. welchii infection. (4 x eyepiece by 16 mm. objective—20 in. bellows.)

this time shows a decided fall in one-half to one hour after injection of the culture. This decrease continues up until death as shown by Chart I.

There is produced at the site of injection a large crepitant swelling in which there is a collection of edematous fluid. In many cases, there is a seepage of this fluid through the needle puncture and sometimes through the pores of the skin over the affected area. The skin becomes discolored, the shading varying from black at the site of the needle puncture to slightly red at the outer edges. This discoloration may extend over the entire side of the breast into which the culture was injected. The subcutaneous connective tissue contains many gas bubbles as well as considerable hemorrhagic edematous fluid. This is usually seen in the connective tissue of the groin in the inoculated as well as the uninoculated side. The muscle fibers are torn apart

by gas pressure and are soft, showing evidence of degeneration and necrosis. Microscopically these changes are seen to have progressed to such an extent that a great many of the cells have been entirely destroyed (Fig. 1).

It is not always possible to recover the organism from the heart-blood immediately after death, especially if the bird has succumbed within a few hours after injection of the culture. In these cases death is apparently due to acute toxemia rather than to bacteremia. However, the organism can usually be readily recovered if the carcass is allowed to remain at room temperature for an hour or more. No difficulty is experienced in recovery of the organism from the site of injection if a bit of the affected muscle tissue is seeded in Hibler medium.

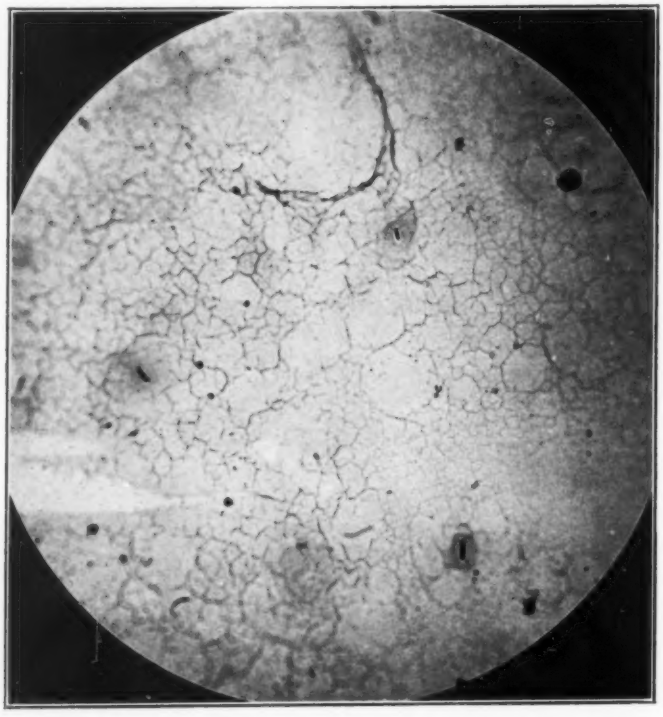


Fig. 2.—B. welchii, blood of pigeon. (4 x eyepiece by 16 mm. objective—20 in. bellows.)

When the organisms are found in the heart-blood they are usually relatively few in number (Fig. 2). They are, however, more numerous in the affected muscle tissue (Fig. 3) and, as seen in smears of the heart-blood, appear somewhat smaller than those in individual colonies taken from 2 per cent glucose agar deep (Fig. 4).

If a bit of the affected muscle is seeded in Hibler medium and smears are made after six hours' incubation at 37° C., the organisms appear as short thick bacilli with an occasional longer one. They are very strongly gram-positive at this stage (Fig. 5). At the end of twenty-four hours' incubation the organisms appear still larger (Fig. 6) and numerous gram-negative forms may be seen. According to some workers this is due to the fact that

the bacilli have lost their power to retain Gram's stain either through age or death. This lack of power to retain Gram's stain is even more marked in older cultures (fourteen days) where only a few gram-positive bacilli are visible.

In its growth, both in the animal body and in culture medium, B. welchii produces an exotoxin which is very actively hemolytic. This toxin was first demonstrated by Bull and Pritchett,[5] who showed that sterile filtrates of cultures were pathogenic to animals, especially guinea pigs, and that serum from animals properly treated contained an antitoxin.

Fig. 3.—B. welchii, infected pigeon muscle. (4 x eyepiece by 16 mm. objective — 20 in. bellows.)

Injection of sterile toxin into the breast muscles of pigeons, which are most uniformly susceptible, produces a condition almost identical to that following the injection of whole cultures with the exception that no gas is produced (Fig. 7).

In the artificial production of toxin, Bengston[7] used a modification of Bull and Pritchett's original medium, consisting of beef infusion broth and chopped fresh veal and containing 0.2 per cent glucose. Adjustment of the reaction of the bouillon to P_H 9.0 to 9.2 prior to the addition of the meat-pieces and sterilization, resulted in a final reaction of approximately P_H 7.4. In my work this final reaction was found to range from P_H 7.2 to 8.0, the variation no doubt being influenced by differences in acidity of the meat-pieces used in the several lots.

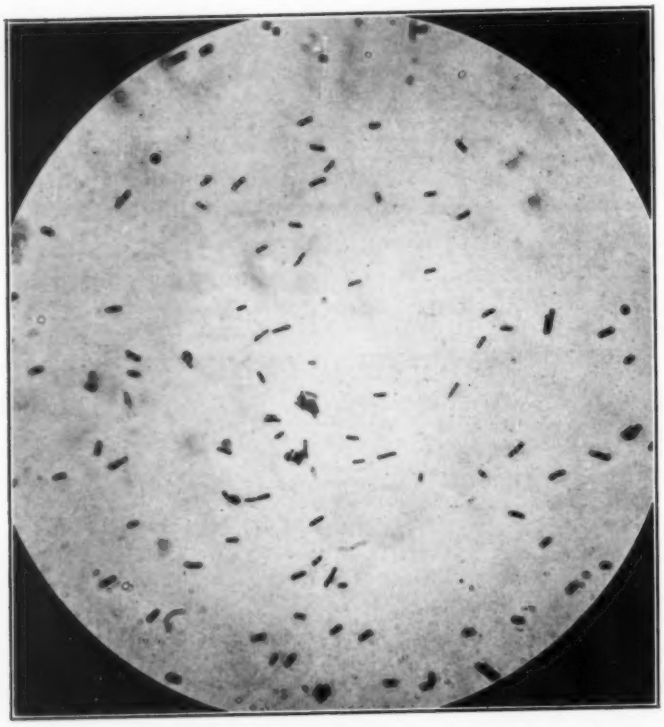


Fig. 4.—B. welchii, colony in 2 per cent glucose agar. (4 x eyepiece by 16 mm. objective — 20 in. bellows.)

Fig. 5.—B. welchii, six hour Hibler medium culture. (4 x eyepiece by 16 mm. objective — 20 in. bellows.)

As in Hibler medium, it was noted that considerable acid was produced during the growth of B. welchii in this veal-piece beef-bouillon medium. In the determination of the hydrogen-ion concentration of a large number of cultures incubated sixteen to twenty-four hours at 37° C., none were found to have a greater acidity than P_H 6.0 to 6.2. These reactions (Table I) were observed for the most part regardless of the initial reaction of the medium. It would seem that when such a reaction is reached, sufficient acid has been

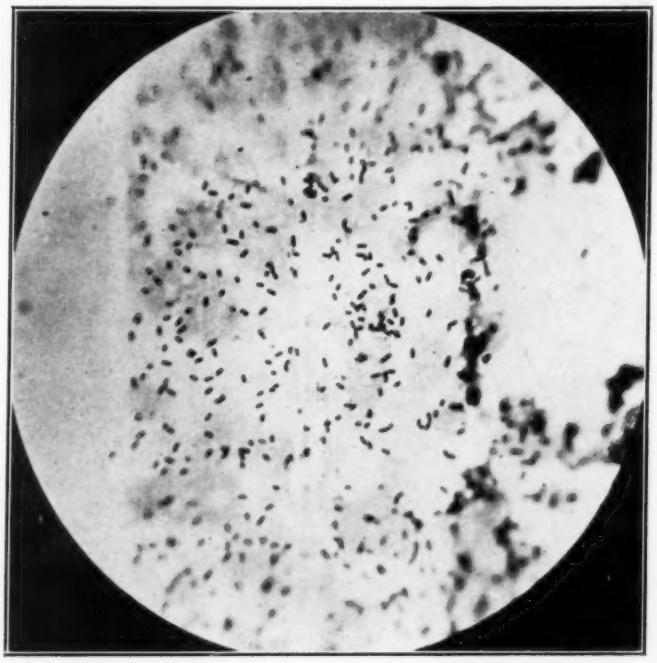


Fig. 6.—B. welchii, twenty-four hour Hibler medium culture. (4 x eyepiece by 16 mm, objective. 20 in. bellows.)

TABLE I

REACTIONS OF CULTURES OF B. WELCHII IN VEAL-PIECE BEEF-BOUILLON MEDIUM

CULTURE	P_H OF MEDIUM	HOURS OF INCUBATION	P_H OF CULTURE
1	8.0	16	6.4
2	7.7	16	6.4
3	7.0-7.2	16	6.2
4	7.6	16	6.2
5	7.6	20	6.4
6	7.6	20	6.6
7	7.8	20	6.6
8	7.8	20	6.2
9	7.2-7.4	21	6.2
10	7.4	21	6.4
11	7.0	21	6.0
12	8.0-8.2	21	6.0-6.2
13	7.8	21	6.2
14	7.4-7.6	22	6.2
15	8.2	22	6.4
16	7.8-8.0	24	6.4
17	7.6	24	6.4
18	7.2	24	6.0

produced to inhibit the growth of the organism, since in no case were these reactions determined until the organisms were partially quiescent.

Bengston has shown that the "fluid toxin of B. perfringens deteriorates to a certain extent just as other fluid toxins, depending on varying conditions of heat, light, reaction and other unknown factors, but that if kept under the most favorable conditions it is reasonably stable." As further evidence

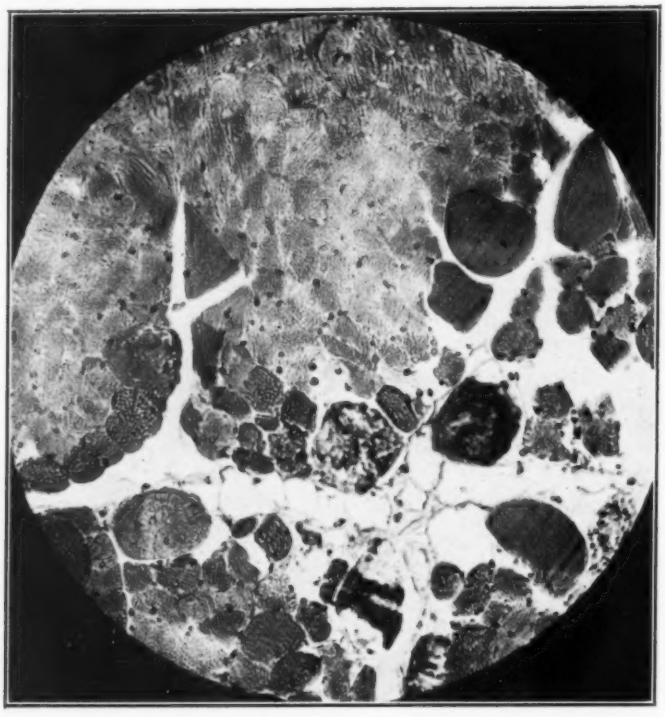


Fig. 7.—Pigeon muscle, effect of B. welchii toxin. (4 x eyepiece by 16 mm. objective — 20 in. bellows.)

TABLE II

DETERMINATION OF TEST DOSE OF PERFRINGENS TOXIN 09251. STANDARD ANTITOXIN P_2–1

TESTS MADE AFTER 60 DAYS' STORAGE AT 2-3° C.							
PIGEON	WT. (GM.)	TOXIN PER 350 GM. C.C.	TOTAL TOXIN C.C.	ANTITOXIN PER 350 GM. C.C.	TOTAL ANTITOXIN C.C.	TOTAL ANTI-TOXIN 1-100 DIL. C.C.	RESULT
295	295	2.73	2.29	0.01	0.0084	0.84	Lived
288	330	2.94	2.76	0.01	0.0094	0.94	Lived
291	285	3.15	2.55	0.01	0.0081	0.81	Died
294	300	3.36	2.85	0.01	0.0085	0.85	Died
TESTS MADE AFTER SEVEN AND ONE-HALF MONTHS' STORAGE AT 2-3° C.							
PIGEON	WT. (GM.)	TOXIN PER 350 GM. C.C.	TOTAL TOXIN C.C.	ANTITOXIN PER 350 GM. C.C.	TOTAL ANTITOXIN C.C.	TOTAL ANTI-TOXIN 1-100 DIL. C.C.	RESULT
89	416	2.73	3.22	0.01	0.0118	1.18	Lived
83	408	2.94	3.41	0.01	0.0116	1.16	Lived
72	394	3.15	3.52	0.01	0.0112	1.12	Died
60	325	3.36	3.11	0.01	0.00928	0.928	Died

of the keeping qualities of the fluid toxin, it has been found that the activity did not change in five and one-half months when stored at 2 to 3° C. (Table II). The bottles employed for storage were of 100 c.c. capacity and after being filled to the neck were corked tightly and sealed with gelatin.

A potent antitoxin can be produced by hyperimmunization of horses with sterile toxin. As a rule these animals are rather sensitive to the action of B. welchii toxin but with care in its administration they become accustomed to its effects and will tolerate relatively large doses. The potency of the antitoxin is measured according to the method of Bengston[7] which is one of the most satisfactory tests for standardization of any of the antitoxins. By this method, the potency of a given antitoxin can be measured with great accuracy, the variable being as small as 0.00025 c.c. per 350 gm. of the pigeon used for test purposes (Table III).

TABLE III

STANDARDIZATION OF PERFRINGENS ANTITOXIN. NO. 2540.

PIGEON	(WT. GM.)	TOXIN PER 350 GM. C.C.	TOTAL TOXIN C.C.	ANTITOXIN PER 350 GM. C.C.	TOTAL ANTITOXIN C.C.	AMT. 1-100 DIL. C.C.	RESULT
245	300	5.56	4.72	0.0025	0.0021	0.21	Lived
236	305	5.56	4.83	0.00225	0.00185	0.185	Dead 19 hr.
239	390	5.56	6.17	0.002	0.0022	0.22	Dead 19 hr.
190	350	5.56	5.56	0.01	0.01		Dead 20 hr.
220	345	0.28	0.98	Standard Control			Dead 20 hr.

SUMMARY

Considerable gas is evolved and there is noted a cloudiness of the liquid when B. welchii is seeded in Hibler medium and incubated at 37° C. Maximum growth follows eight to twenty-four hours' incubation. Acid is produced, the reaction of the cultures ranging from P_H to 6.0 to 6.4 although the initial reaction of the medium may have been P_H 7.6 or even more alkaline.

In shake cultures in a 2 per cent agar medium containing 2 per cent glucose, sufficient gas may be evolved to shatter the agar. The colonies are essentially lenticular and, if the seeding has been heavy, are small. When the seeding has been light, the colonies are larger and often appear broken along one edge.

Pigeons are for the most part uniformly susceptible to B. welchii and its toxin. Crepitant swellings with a collection of edematous fluid follow intramuscular injection of pure cultures. Death may follow in from two to twenty-four hours after administration of a virulent culture. On postmortem there is seen degeneration and necrosis of the muscle tissue which is discolored and may be torn apart by gas. Collections of edematous fluid in the subcutaneous connective tissue occur. Injections of sterile toxin are followed by practically the same changes as when whole cultures are injected, with the exception that no gas is produced.

The appearance of the organisms in cultures in Hibler medium change with age, being on the whole smaller and strongly gram-positive when young

but larger in older cultures. In the latter, a greater percentage of the organisms may be gram-negative.

No reduction in activity of fluid toxin was noted after five and one-half months' storage at 2-3° C. in sealed amber bottles.

The potency of the antitoxin can be measured with great accuracy, the variable being as small as 0.00025 c.c. per 350 gm. of the pigeon used for test purposes.

REFERENCES

1. Keen's Surgery: **8**: 1922 Ed., p. 730.
2. King, Walter E.: Gas Bacillus Infection in Civil Life, Am. J. Surg., August, 1931.
3. Simonds, J. P.: Studies in Bacillus Welchii, With Special Reference to Classification and Its Relation to Diarrhea, Monog. Rockefeller Inst. Med. Res. No. 5, 1915.
4. Bull, Carrol, G., and Pritchett, Ida W.: Identity of the Toxins of Different Strains of Bacillus Welchii and Factors Influencing Their Production in Vitro, J. Exper. Med. **26**: 867, 1917.
5. Bull, Carrol, G., and Pritchett, Ida W.: On the Production of B. Welchii Toxin, J. Exper. Med. **26**: 119, 1917.
6. Goss, L. W., Barbarin, Rhea, and Haines, A. W.: Some Characteristics of B. Chauvoei, J. Infect. Dis. **29**: 615, 1921.
7. Bengston, Ida A.: Standardization of Gas Gangrene Antitoxin, Hyg. Lab. Bull. No. 122, 13, 1920.

ARTERIOSCLEROSIS OF THE CORONARY ARTERIES: A CLINICOPATHOLOGIC STUDY*

By Don C. Sutton, M.S., M.D., and W. W. Brandes, M.D., Ph.D., Chicago, Ill.

THIS study was originally begun by one of us (D.C.S.) as a study of 340 cases in which the gross findings at autopsy were those of sclerosis of the coronary arteries. These cases were selected from the autopsy records of Cook County Hospital. By a study of the autopsy reports and a correlation of the clinical records it was hoped to orient ourselves as to the relationship of coronary artery disease to the clinical evidence of that disease. Added to the original series are 49 cases taken at random from the autopsy records of Evanston Hospital, these being selected if any pathology of the coronary arteries or myocardial scars were noted. Further gross and microscopic studies were made by Dr. Brandes.

There are three possible causes of interstitial changes in the myocardium, namely:

1. Arteritis (acute) with fibrosis spreading outward from a periarteritis or an obliterating endarteritis, producing anemia or infarction.

2. Interstitial proliferation from toxic or bacterial action.

3. Primary muscle degeneration; (a) from anemia due to partial arterial occlusion, (b) from infarction, (c) from toxic or bacterial muscle degeneration.

There is abundant evidence of acute arterial changes occurring in the coronary as well as systemic arteries in the course of acute infectious diseases.

Therese,[1] Sinnitzky,[2] Wiesel,[3] Weisner,[4] Frothingham,[5] have found them in diphtheria, scarlet fever, pneumonia, smallpox, typhoid fever, influenza, tuberculosis, pyocyaneus, streptococcic infections, and syphilis.

Rabe,[6] Cowan,[7] Coombs,[8] Klotz,[9] and Gray and Aitken[10] have noted almost constant arterial changes in rheumatic fever. Bakwin[11] and Stoerk and Epstein[12] found atheromas in the coronary arteries in almost every patient dying of influenzal pneumonia. Clawson[13] found myocardial scars in 80 per cent of cases of acute rheumatic endocarditis, 65 per cent in recurrent rheumatic endocarditis, and in 53 per cent of old valvular lesions. Many have believed these arterial changes to be the precursors of arteriosclerosis.

Jores[14] has emphasized the difficulty of deciding in every case, even in the presence of round-cell infiltration, whether or not the intimal thickening is of inflammatory origin.

Brooks[15] found evidence of syphilitic involvement of the coronary arteries in the majority of 50 patients dying of cardiovascular syphilis. The changes

*From the Medical Service of Cook County Hospital, and the Department of Pathology, Northwestern University Medical School.

Received for publication, March 17, 1931.

observed in acute arteritis are intimal thickening, even with complete occlusion of the artery, so that even acute myocardial lesions may be, and we believe are, secondary to the arterial obstruction.

Chronic Arteriosclerosis.—Walkoff[16] finds that there is a tendency to greater proliferation in the intima of the coronary arteries than in systemic arteries of the same caliber. Thorel[17] calls attention to the great variations in myocardial changes, even in extensive narrowing of the coronary vessels, and believes a partial explanation to be the variations in anastomosis of the various branches. He further suggests the effect upon the smaller branches of a severe sclerosis of the larger vessels; if it were less in the finer vessels the heart muscle could still receive sufficient blood.

THE PRESENT STUDY

The first group of cases studied consists of 340 autopsy records in which gross coronary artery sclerosis is noted in the records. These are taken from the autopsy records of Cook County Hospital, from 1925 to 1928, and are from a total of 3,040 autopsies. The incidence of coronary artery sclerosis in this series is 11.1 per cent. It is obvious that this group includes a great variety of primary pathologic conditions, and that most of the patients were in the hospital for other than cardiovascular symptoms. (Table I.)

TABLE I

CASES FROM COOK COUNTY HOSPITAL AUTOPSY RECORDS

Coronary artery sclerosis	340	
With occlusion	72	
With thrombosis and infarction	43	12.6%
With gross myocardial scars	88	29+ %
Syphilis (positive Wassermann 20)	60	
Aortitis	53	15.5%
Aneurysm	7	
Peripheral arteriosclerosis	113	32.2%

Gross scars of the myocardium occurred in 88, or 29+ per cent, not including the scars of infarcts. The left coronary artery, or one of its branches, was recorded as being thrombosed twenty times, and the right coronary artery eight times. The artery involved was not recorded in the remaining 23 cases of infarction.

For a more accurate and detailed study, a series of 49 autopsies at the Evanston Hospital were selected because arteriosclerosis had been found either macro- or microscopically. These were reexamined and carefully studied for arterial, interstitial and myocardial changes. One of the earliest observations noted was the frequency with which a coronary artery showing no obvious evidence of arteriosclerosis revealed on close inspection sclerosis about the ostea of the smaller branches, which was often sufficient to narrow definitely the arterial opening. The early age at which definite arteriosclerotic changes were found was surprising, the youngest being five years. In a boy twelve years of age, who had had an endocarditis lenta for three years, definite gross sclerosis of the larger coronary arteries was evident.

Macroscopic sclerosis of the coronary arteries was present in 35 cases, varying from 14 with slight sclerosis, 13 with moderate, to only 8 with severe sclerosis (Table II). Grossly, myocardial fibrosis was found in only 6 cases. Microscopically, sclerosis of the smaller arteries was found in 42 cases; 9 slight, 23 moderate and 10 severe. Fibrous scarring of the myocardium was present in 40 cases; 8 were slight, 27 moderate and 5 severe. The amount of fibrosis upon the average is in direct ratio to the amount of vascular damage.

TABLE II

EVANSTON HOSPITAL GROUP—49 CASES

Macroscopic				
Coronary artery sclerosis			35	72.9%
Slight			14	40.0%
Moderate			13	37.0%
Severe			8	22+ %
None			13	
Myocardial scars			6	12.5%
Microscopic				
Vessel Sclerosis			Myocardial Scars	
Slight	9	18.6%	8	15.5%
Moderate	23	47.8%	27	56.2%
Severe	10	18.0%	5	10.2%
Total	42	87.4%	40	83.3%
Muscle edema				10
Muscle necrosis				4
Syphilis				1

EXPERIMENTAL STUDIES

In the course of other investigative work the heart muscle of dogs was studied microscopically at varying periods after the following procedures:

1. The ramus descendens anterior sinister was dissected free and a portion of one centimeter cauterized with phenol and washed with alcohol.

2. The ramus descendens anterior sinister was clamped tightly, and momentarily, two or three times.

A few hours after pinching the artery, myocardial sections from the area of the distribution of the vessels show cloudy swelling of the muscle fibers.

Some of the smaller arteries and capillaries are distended with blood. There is no evident hemorrhage or inflammatory reaction. The muscle fibers show an irregularity in staining, some are pink and some are very pale. Some fibers are slightly swollen and the cross striations indistinct.

Ten days after pinching the artery, although the artery at the point of injury was patent to injected fluid, it showed a thrombosis partially occluding the lumen. In a section of myocardium there are small areas of necrosis with cellular infiltration. In a cauterized specimen after three days the muscle fibers are swollen and the interstitial spaces narrowed or obliterated. Von Redwitz[18] and Farmachidis[19] have observed fatty degeneration and capillary hemorrhages in the myocardium in cases of experimental arteriosclerosis.

RELATION OF HYPERTROPHY

Eyster[20] believes that the most important factor leading to cardiac hypertrophy developing in organic cardiac or vascular disease is not due to increased work, but is the reaction of injury that results from abnormal stretching of the muscle in the initial period of overload, as the lesion develops.

In the Cook County Hospital series ventricular dilatation and hypertrophy occurred as follows: (Table III.)

TABLE III

THE OCCURRENCE OF HYPERTROPHY AND DILATATION OF THE VENTRICLES

	DILATATION ONLY	HYPERTROPHY ONLY	BOTH HYPERTROPHY AND DILATATION
Right ventricle	5	33	4
Left ventricle	0	59	5
Both right and left ventricle	7	89	23

Dilatation alone of the right ventricle only occurred five times, of the left ventricle only, none, and of both right and left ventricles seven times.

Right ventricle hypertrophy only, occurred 33 times, of the left ventricle only, 59 times, and of both the right and left ventricles, 89 times. Both dilatation and hypertrophy of the right ventricle only, occurred 4 times, of the left ventricle only, 5 times, and of both right and left ventricles, 23 times.

Clinical Studies.—In the Cook County Hospital series the number of males is 254, of females 86. This figure is in keeping with the large number of males in proportion to females admitted to the hospital. In the Evanston Hospital group they are evenly distributed, 24 males and 25 females. The ages in both groups ranged from the second to the ninth decade. In the Evanston group three occurred in the first decade. (Table IV.) The incidence increases with each decade, reaching its height in the seventh decade, then falling off sharply.

TABLE IV

AGES

COOK COUNTY		EVANSTON	
10-19 years	2	5- 9 years	3
		10-19 years	3
20-29 years	13	20-29 years	1
30-39 years	27	30-39 years	4
40-49 years	59	40-49 years	7
50-59 years	69	50-59 years	7
60-69 years	88	60-69 years	14
70-79 years	40	70-79 years	6
80-89 years	16	80-89 years	2
Total	314		47
Females	86		25
Males	254		24

The clinical records of 205 of the County Hospital cases were tabulated (Table V). Seven cases were discarded because no cardiac symptoms were recorded. In this group of 198 cases, dyspnea is recorded 102 times, or 51.5 per cent. Precordial pain is recorded 35 times, 18.1 per cent, 11 of which were

diagnosed as angina pectoris. Edema is recorded 81 times, or 40.8 per cent. Systolic murmurs without valve lesions were described 67 times, or 33.8 per cent. Irregularities were mentioned 71 times, or in 35.8 per cent.

TABLE V

TOTAL CLINICAL RECORDS	205	
NO CARDIAC SYMPTOMS	7	
	198	
Dyspnea	102	51.5%
Precordial pain	35	18.1%
Angina pectoris	11	
Edema (peripheral)	81	40.8%
Systolic murmurs without valve lesion	67	33.8%
Irregularities (all types)	71	35.8%

DISCUSSION

The relationship of coronary artery sclerosis to myocardial scarring from this study evidently depends upon whether the analysis of material rests only upon gross pathology or on both gross and microscopic changes.

That extensive changes in both the lumen of the smaller branches of the coronary arteries and similar interstitial changes may be present microscopically with little or no gross changes present, has been quite evident. That such changes can occur as the result of only moderate changes in coronary flow has been shown in the experiments cited (which will be reported in detail later).

Although obvious scars were shown in only 29 per cent of the Cook County Hospital series, symptoms such as dyspnea occurred in 51.5 per cent. It appears then that either microscopic scars may be the cause of symptoms of circulatory failure, or that there are other causes not revealed by pathologic anatomy. Clawson[13] has said that "so-called myocarditis is usually a condition of the myocardium, probably fatigue, which is not manifested anatomically."

The statement of Claude Bernard,[21] that "there may be sufficient blood supply to an organ for its nutrition and yet not sufficient for its function" appears more applicable as an explanation.

Scarring of the myocardium, either infectious or degenerative, either grossly or microscopically, is the result of sufficient loss of blood to the scarred area to result in necrosis of muscle fibers or practically infarctions. The function of such a myocardium depends not upon the effect of the scars but the nutrition supplied to the remaining normal myocardium. The amount of blood flow to the myocardium depends upon the coronary artery anastomoses. Therefore, an extensively scarred myocardium may have sufficient collateral circulation for normal function, whereas a slightly scarred myocardium may fail because of the lack of such anastamoses. In other words, myocardial failure in arteriosclerosis of the coronary arteries depends upon myocardial anemia. The same is true in regard to precordial pain (Sutton and Lueth).[22]

In this connection it is important to reiterate the importance of localized arteriosclerotic patches at the ostea of the smaller branches of the coronary

arteries similar to those occurring at the orifices of the coronary arteries in the aorta. This may serve to explain many cases of angina pectoris in which the coronary arteries have been reported as normal.

CONCLUSIONS

1. Arteriosclerosis of the coronary arteries may often be present without macroscopic scarring of the myocardium.

2. In microscopic arteriosclerosis of the vessels concomitant myocardial scarring is practically constant.

3. Myocardial scars from experimental evidence and microscopic study are believed to be due to occlusion of the involved arteries.

4. Myocardial function in arteriosclerosis of the coronary arteries depends upon blood flow and not scarring, and varies with the degree of anastomosis present.

REFERENCES

1. Therese: Etude anatomo-pathologique et experimentale sur les arteritis secondaires aux maladies infectieuses, These de Paris, 1893.
2. Sinnitzky: Über die Haufigkeit von arteriosklerotischen veranderungen in der aorto jungenlichen Individuen, Ztschr. f. Hielk. **24**: 177, 1903.
3. Wiesel, J.: Über Erkrankungen der Koronararterieñ Verlaufe akuter Infektious Krankheiten, Wein. klin. Wchnschr. **19**: 723, 1906.
4. Wiesner: Über Veranderungen der Koronararterien der Infektious krankheiten, Wein. klin. Wchnschr. **19**: 725, 1906.
5. Frothingham, C.: The Relation Between Acute Infectious Diseases and Arterial Lesions, Arch. Int. Med. **8**: 153, 1911.
6. Rabe, A.: L'etude des Lesions des Arteries dans les Infections rheumatismales, Presse méd. **10**: 927, 1902.
7. Cowan, J. M.: The Heart in Acute Disease, J. Path. & Bact. **9**: 88, 1903.
8. Coombs, C.: Rheumatic Myocarditis, Muenchen. med. Wchnschr. **54**: 1057, 1907.
9. Klotz, O.: Rheumatic Fever and Arteries, Trans. Assoc. Am. Phys. **27**: 181, 1912.
10. Gray-Aitkin: Late Gross Lesions in the Aorta and Pulmonary Artery Following Rheumatic Fever, Arch. Path. **8**: 451, 1921.
11. Bakwin, H.: Gross Pathology of Influenzal Pneumonia in France, Am. J. M. Sc. **159**: 435, 1920.
12. Stoerk, O., and Epstein, E.: Über Arterielle Gefassverunderungen bei Grippe, Frank. Ztschr. f. Path. Weisb. **23**: 163, 1920.
13. Clawson, B. J.: Myocarditis, Am. Heart J. **4**: 1, 1928.
14. Jores, L.: Henke and Lubarsch. Hanb. **2**: 607, 1924.
15. Brooks, H.: Am. J. Syph. **5**: 217, 1921.
16. Walkoff: La Structure histologique des arteries, Abstr. Maladies du Coeur **17**: 7, 1924.
17. Thorel, C.: Pathologie du Kreislauf organe Ergebuisse der Allgemeine Pathologie und Pathologischer Anatomie, Lubarsch u. Obstertag, 195, **17**: 494, 464.
18. Von Redwitz, E.: Der Einfluss der Erkrankungen der Koronar arterien auf die Kerzmuskulatur mit besonderer Bernschitigung der Chronischen Aortitis, Virchows Arch. **197**: 1910.
19. Farmachidis, C. B.: Einfluss der Arteriosklerose auf das Myocard, Deutsch. med. Wchnschr. 1912, L B S 1705.
20. Eyster, J. A. E.: Experimental and Clinical Studies in Cardiac Hypertrophy, J. A. M. A. **91**: 1881, 1928.
21. Claude Bernard: Quoted by Huchard, Henri, Traite Clinique des Maladies du Coeur. Ed. 3, Paris, 1899.
22. Sutton, D. C., and Lueth, Harold C.: Pain, Arch. Int. Med. **45**: 827-867, 1930.

CASE REPORT OF EXTENSIVE ATROPHY OF SUBCUTANEOUS FAT FOLLOWING THE REPEATED INJECTIONS OF INSULIN*

By John R. Williams, M.D., Rochester, N. Y.

DURING the past two or three years reports have been appearing in medical literature of the various complications which result from the repeated use of insulin. Allergic reactions have been commonly noticed because they give rise to distressing symptoms from which the patient demands relief. Atrophy of the subcutaneous fat, however, is a painless complication and one which the patient is very apt to overlook. The main reason for reporting this case is to emphasize the necessity of the frequent inspection of patients who are using insulin to make sure that there are no untoward complications. This is particularly necessary when it is considered that the great majority of patients now using insulin are doing so following a very brief instruction in a doctor's office. Many of these will use insulin continuously once or twice daily, oftentimes for periods of months or years, without reporting to a physician for examination. A number of such cases have come to my notice.

In the well-regulated diabetic clinics patients are instructed not to inject insulin into one site a second time until an interval of at least two weeks has passed. Unfortunately even the most intelligent patients will disregard these precautionary instructions. When one site is repeatedly injected the sense of pain is lost. The patient discovers this and to escape the momentary sting of the needle puncture will use one small area repeatedly. I have two such cases in young women who stoutly oppose the use of any other part of the body than a small portion of the thigh where the injections have been confined to an area of less than one square inch. In each case the skin has become hard and indurated. The condition was discovered because of the great variation in their glucose utilization. In all probability the greater part of the insulin was not being absorbed. It was also discovered that a portion of the insulin leaked from the scar back along the track of the needle. In neither of these cases had fat atrophy occurred but definite scar formation was evident.

A great majority of patients inject insulin into the thigh for the reason that this part of the body is most convenient and less easily irritated. When patients report for a check examination it is the common practice to question and examine them for evidences of vascular disturbances, reactions, edema, gastrointestinal, and visual disturbances. Because of the apparent safety or innocuousness of the hypodermic therapy clinicians rarely inspect the site of the insulin injection. In view of the case reports of Barborka,[1] Rabinowitch,[2]

*From the Highland Hospital.
Received for publication, February 12, 1931.

Fischer,[3] Avery,[4] and others, the necessity of a physical inspection of the part of the body used for the insulin therapy becomes necessary. The following case in my own service well illustrates the possibilities of such an inadvertence. The patient, a female school-teacher aged forty-four years, is a rather severe diabetic with an insulin coefficient of approximately 25. She has been under observation since April 3, 1928, taking most of the time, two doses of insulin daily. From April to September, 1930, inclusive, the patient took two doses of insulin daily, varying from five units to ten units. Since that time she has been taking twenty units daily in doses of ten units. The areas of atrophy began to appear about July 1, 1930, and continued to develop up to November first when new sites for injection were selected and greater care exercised. No evidence of atrophy is occurring in these areas and none was

Fig. 1.—Atrophy of subcutaneous fat following repeated insulin injections.

evident on inspection. On January 10, 1931, during the course of an examination, the patient casually remarked about the hollows in her thighs. Examination revealed a marked atrophy of the subcutaneous fat on both thighs, as is clearly shown in the accompanying photograph. These atrophic areas are irregular in shape and vary from 8 to 15 cm. in diameter. The long diameters extend up and down the thigh. The cavities are from 2 to 3 cm. deep. In each thigh there may be seen three of these great cavities and one of smaller size. The fat seems to be completely excavated. The skin is free. The muscles and blood vessels can be felt distinctly beneath. There is no sign of soreness or inflammation. The patient is in good health and has not been inconvenienced by the atrophy. For the past two months the patient has been injecting insulin in the upper and outer parts of the thigh. Similar phenomenon was noticed several months ago in a girl twelve years of age who

was taking four small doses of insulin daily. Both mother and patient were cautioned about the importance of selecting fresh areas for each injection and the atrophy in this case has disappeared, being replaced by fresh fat.

The cause of this very unusual atrophy is not known. Both Fischer and Rabinowitch have studied the problem with some care. The points these writers emphasize are that it occurs mainly in females, that it is a loss of fat with fibrosis but with no evidence of infection, that lipase is not a factor, that it is possible the insulin injures the membrane that covers the fat cells releasing fat globules, these in turn acting as foreign bodies and resulting in the formation of histocytes which take on lipophagic activity. Another and very suggestive explanation is that it is a low-grade inflammation due to the repeated injections of insulin causing the disappearance of the soft areolar tissue and its replacement by scar tissue. Nerve injury with consequent atrophy is a possibility. The point most to be emphasized is that physicians prescribing insulin should admonish their patients about the necessity of constantly changing the site of injection, never using the same area twice in a shorter period than two weeks. Indeed, the propriety of permitting untrained diabetic patients to use insulin is open to question.

REFERENCES

1. Barborka, C. J.: Fatty Atrophy From Injections of Insulin, J. A. M. A. **87**: 1646, 1926.
2. Rabinowitch, I. M.: Case of Extensive Atrophy of Subcutaneous Fat Following Injections of Insulin, Canad. M. A. J. **21**: 67, 1929.
3. Fischer, A. E.: Frequency of Atrophy of Subcutaneous Fat Following Injection of Insulin, Am. J. Dis. Child. **38**: 715, 1929.
4. Avery, H.: Insulin Fat Atrophy a Traumatic Atrophic Panniculitis, Brit. M. J. **1**: 597, 1929.

ELECTROCARDIOGRAPHIC STUDIES ON CHICK EMBRYO HEARTS*

I. A Technic for Recording Electrical Changes in Isolated Chick Embryo Hearts

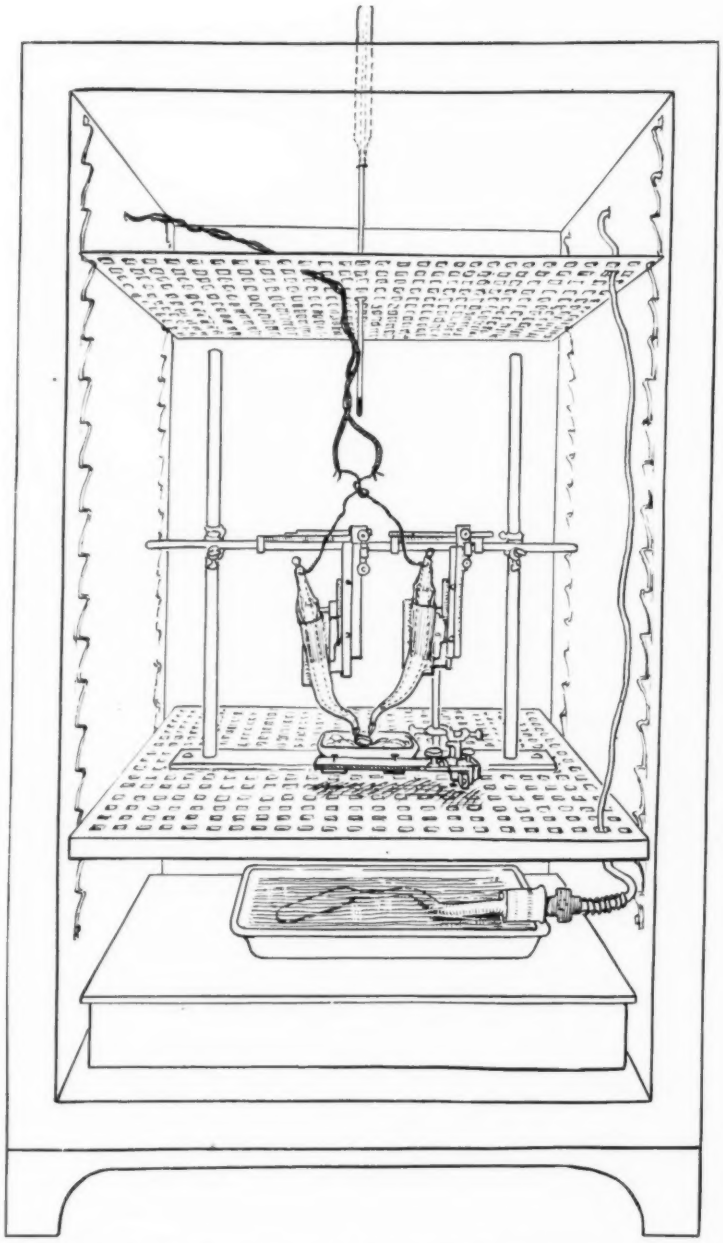
By Herbert Pollack, M.D., Boston, Mass.

THE isolated intact warm-blooded heart under suitable conditions lends itself to many experimental procedures. The literature contains a few references to the normal electrocardiograms of developing chick embryos (Cluzet et Sarvonat, 1914); (Wertheim-Salmonson, 1913); (Spadolini and de Georgis, 1921); (Sands, 1929). There is, however, a surprising lack of reports on experimental alterations of the normal. It was thought that a technic of recording the electrical changes in the isolated warm-blooded (Pollack, 1930) heart would offer possibilities of determining the direct effects of many drugs. This should alleviate much of the criticism directed toward the work on cold-blooded hearts as not pertaining to the warm-blooded. The plan of this work is to study (a) the functional changes as recorded by the electromyograms; (b) chemical changes by subsequent analyses; and (c) histopathologic changes.

Toward this end the following technic was devised and has proved to work satisfactorily. Fertile chicken eggs are incubated to the desired stage. The embryos are removed from the shell and pinned out on a paraffin block. The heart is dissected out carefully, leaving a fairly long stalk of great vessels and some tissue. This avoids injury to the auricles and allows for handling. With practice the time taken for this procedure is negligible. The shock during this operation may sometimes cause the heart to stop beating temporarily. The normal rhythm is usually reestablished when the heart is placed in a warm incubator. The receptacle for the heart is made of an oblong glass dish about 2 cm. deep. This is filled with paraffin. A hole is excavated in the paraffin approximately $2 \times 1 \times 1.5$ cm. The dish is cemented to an ordinary microscopic slide which is set on a mechanical stage.

The electrodes for leading the current from the heart must be chosen for (1) nonpolarizable character, (2) low resistance, and (3) small surface for contacts. The type finally chosen as being most suitable for this work is a modified zinc-zinc sulphate cell. An ordinary culture tube is drawn to a capillary about three inches from the mouth. The constricted portion between the capillary tip and body of the tube is bent out at about a 60° angle. The capillary tip is then bent down in the complementary angle at a point where it extends beyond the outer wall of the tube, and cut to leave a tip of a few millimeters. This is done to allow approximation of the tips of the two

*From the Laboratory of Pathology, Palmer Memorial Hospital.
Received for publication March 4, 1931.



View of Incubator Showing Construction and Arrangement of Electrodes

electrodes. A glass slide is cemented to the body of the tube with DeKhotensky for fixation to mechanical stages. The capillary tip is threaded with several thicknesses of cotton string. These should be snugly packed in the capillary opening. Too tight a fit interferes with capillarity, causing poor wetting, which increases the resistance. The thread should be run well up into the body of the tube. The outside string is cut about 0.5 cm. from the opening and wound with a spiral thread. Sodium chloride solution (0.9 per cent) is used to saturate the string, which serves as the immediate contact with the heart. The tube is filled with a thin paste of kaolin and saturated zinc sulphate. The zinc poles are pushed as far down into the tube as possible. By grinding and bending the distal ends they may be fitted into the curves. The tubes are stoppered by corks bored for the poles and sealed with thick celloidin. The electrodes have a resistance varying from 2000 to 4000 ohms. They can be used for constant recording over periods of several hours without showing an appreciable amount of polarization or resistance changes. Owing to the action of the zinc sulphate on the cotton, it is advisable to renew the filling every eight weeks.

In order to maintain the hearts at proper temperature, the electrodes were set up inside an incubator. An iron bar three inches wide, three-quarters of an inch thick, and long enough to reach across the incubator is tapped out for three vertical one-half inch steel rods. The two taller rods are set about two inches from each end, and a shorter one set halfway from the center to one end. Another rod is clamped rigidly across the two end vertical ones at the desired height. The base bar is rigidly clamped to the incubator shelf. The mechanical stages with the electrodes are clamped to the crossbar. The mechanical stage with the chamber is clamped to the middle short vertical bar by means of a short side arm. This gives a three dimensional range of motion in placing the electrode in relation to the heart.

The use of an electrolyte medium for bathing the heart introduces a very important factor in the electrical circuit. Any saline medium flooding the chamber acts as a shunt across the electrode tips and tremendously reduces the recorded voltages. The fact that the cotton tips of the electrodes have the same resistance as the medium in which they are bathed prevents any efficient alterations at that place. There are two ways around this difficulty: (1) to use a "dry" heart, or (2) develop a device to amplify the recorded potentials.

For the work on the developmental aspect of the electrocardiogram of the embryo the dry heart was used. For the experimental work in determining the effect of drugs, where a saline medium is necessary, an amplifier was developed. A description of this will soon be published.

Alternating currents are one of the most disturbing factors encountered in the development of the technic. These can be reduced to a minimum by complete shielding. If an electric incubator is used, all wires must be entirely disconnected by removing the plugs from the sockets during the time when the string galvanometer is in the circuit. If the lighting current is of the A.C. type, it is preferable not to have any bulbs lit in the incubator room.

The cable to the string galvanometer is run directly into the incubator and the wires connected to the zinc poles. The connections are waterproofed with several coats of Ambroid cement. The danger of exposed unions of two dissimilar metals lies in the condensation of water vapor and the setting up of currents between the copper and zinc. This results in a wandering string. The metal coating of the cable must be grounded at one end at least.

When the bare heart is used proper moisture conditions are maintained by saturating the air of the incubator with water vapor. A shallow enamel pan of the type used in photography is placed on the bottom of the incubator. This is filled with water. An immersion "Hot Point" heater is set in the water and the cord run out through a ventilating hole in the wall of the incubator.

The cooling of the incubator when the heating currents are off is minimized if the pan of water has been brought to a boil before disconnecting the current.

Three leads from the heart are feasible in embryos over four days. The attempt was made to keep them referable to the usual clinical leads. Lead α taken across the two auricles, lead β is taken with one electrode on the right auricle and the other at the apex, γ is left auricle and apex. The records of various stages are consistent when taken at different times. Minor variations in the position of the apical electrodes in leads β and γ do not affect the fundamental picture.

SUMMARY

A technic is described for obtaining electrocardiographic tracings from the isolated chick hearts. The various difficulties which may be encountered are outlined with instructions for avoiding them.

REFERENCES

1. Cluzet et Sarvonat: L'électro-cardiogramme de l'embryon de poulet, J. de physiol. et de path. gén. **16**: 802, 1914, 15.
2. Wertheim-Salmonson, J. K.: Das Elektrokardiogramm von Hühnerembryonen, Pflüger's Arch. **153**: 553, 1913.
3. Spadolini, I., and di Giorgio, A.: L'elettrocardiograma embrionale, Arch. di Fisiol. **19**: 479, 1921.
4. Robb, Jane Sands: The Elemental Character of Embryonic Electrocardiograms, Abstracts XIIIth International Physiological Congress, p. 227, 1929.
5. Pollack, H.: A Technique for Recording Electrical Changes in Isolated Chick Embryo Hearts, Proc. Soc. Exper. Biol. & Med. **28**: 61, 1930.

ELECTROCARDIOGRAPHIC STUDIES ON CHICK EMBRYO HEARTS*

II. An Amplifying Device for Use With a String Galvanometer

By Herbert Pollack, M.D., Maurice Dionne, A.B., and Elwood Schafer, Boston, Mass.

IN A PREVIOUS paper[1] (Pollock, 1931) mention was made of the necessity for an amplifying device in certain experiments in this series. For that purpose the vacuum tube amplifier described in the following article was found to be acceptable.

For this work an amplifier must have an exceedingly uniform frequency-response characteristic as well as the necessary over-all gain, in order that the input wave-forms produced by the heart voltages shall be reproduced at the output of the amplifier with the greatest possible fidelity. Furthermore, the width of the frequency spectrum which must be amplified with uniform gain is unusually wide due to types of waves which must be dealt with. In the ideal case, an amplifier should transmit frequencies down to zero (that is, a steady constant voltage) and up to 20,000 or 30,000 cycles per second in order to reproduce accurately both the small, slow rise of the T-wave and the sharply peaked QRS complex. The former wave-form is in some cases nearly a constant value which corresponds to a very low frequency and the latter owes its sharp peak to the presence of a large number of higher harmonic frequency components.

In practice, it is impossible to reach either the lower or upper limits desired. However, it is possible to construct an amplifier which will reproduce records which are but slightly distorted. The distortion is probably less than that unavoidably imposed by the string galvanometer itself.

The circuit chosen for this amplifier was the so-called "direct-coupled" or "pure resistance coupled" amplifier. A series of vacuum tubes can be so connected that the DC plate current normally flowing through a resistor in the plate circuit of any tube produces a voltage drop across this resistor. This voltage drop can be made to serve as the grid biasing voltage of the succeeding tube if the proper choice of tubes and resistors is made. A schematic diagram of this circuit is shown in Fig. 1.

With such an arrangement any small variation in the potential applied to the grid of the first tube will cause a change in the plate current of this tube with a resultant change in the voltage drop across its plate resistor. Hence, any change in the voltage drop across the first resistor will cause a similar change in the grid potential of the second tube. This same action is repeated for each stage, with a voltage step-up or multiplication taking place

*From the Laboratory of Pathology, New England Deaconess Hospital and the Lahey Clinic.

Received for publication, March 4, 1931.

in each vacuum tube. Due to the cascade arrangement each successive amplification acts upon the result of all former amplifications so that the over-all gain increases rapidly with the number of tubes.

For the purpose of amplifying the embryo heartbeat voltages an amplifier with a gain of several hundred was found to be sufficient. This amplifier employed three Radiotron 201-A type tubes in the circuit shown in detail in Fig. 2. Three separate blocks of 48 volt storage B batteries were used, with a 6 volt storage cell to light the filaments. Wire wound resistors were used in the plate circuits to eliminate a possible source of undesirable variation in resistance as is common with other types. Those used were Electrad Type C

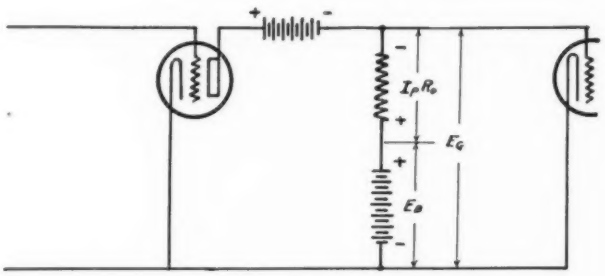


Fig. 1.

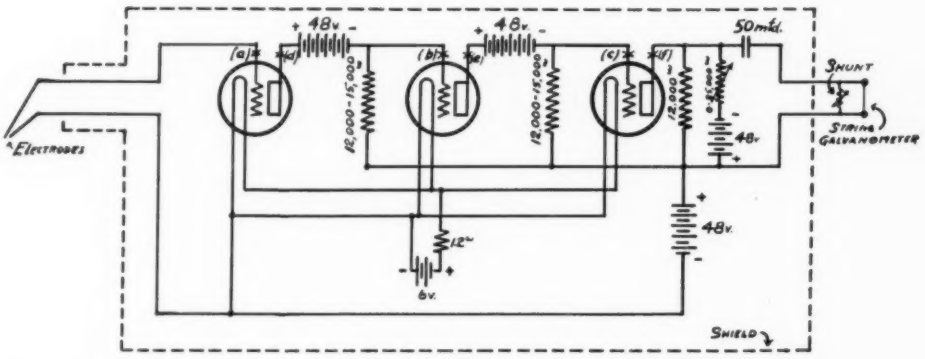


Fig. 2.

25,000 ohm wire wound resistors. Tube sockets which give positive contact to the pins of the tubes must be used. An even better method is to solder the connections directly to the pins themselves. Tubes must be chosen which have a high and steady electron emission and are as free as possible from microphonic effects.

If no special apparatus is available for testing the tubes a makeshift can be made by using the amplifier with a meter in the plate circuit of the first tube. A tube with good emission should not show an appreciable decrease in plate current if the filament voltage is decreased to 3.5 volts. A microphonic tube can be detected by tapping it and noting the jump in the plate current. These measures are only relative but the worst tube can be picked out in this

manner and placed in the last stage so that its microphonic variations will be acted upon by as little amplification as possible.

The filament circuit should not contain a rheostat but should be connected directly to the battery if within 5 per cent on the rated 5 volts can be so obtained. If not, a fixed resistor of extra high current carrying capacity should be used to secure the correct 5 volts on the filaments. The reason for such a large resistor is to prevent any slow changes due to the heating, from taking place. For the same reason connections to the battery should be of fairly heavy wire and well soldered to the battery itself. B battery connections should likewise be soldered.

Shielding the amplifier against stray voltages from the AC lighting circuits and telephone lines is absolutely necessary and the more complete this shielding is made the better. Particular care must be taken to shield the input wires leading from the incubator to the amplifier since very small voltages induced in them will produce string fluctuations proportionate to the heartbeats themselves. Under most conditions a ground connection should be made to the shielding, although it is well to try the amplifier without it in some installations.

Supporting the amplifier on springs will greatly reduce the effect of microphonic tubes. The most convenient method of doing this is to mount tubes and resistors on a small board which can be suspended in the shielding box by damped springs. Wrapping the tubes with cotton and damping the period of the springs with cotton placed inside each spring helps to produce a quiet amplifier. All lead wires to this panel must be extremely flexible. Supporting the shield itself on cotton or pillows gives an added freedom from these disturbances.

To prevent variations at the electrodes themselves it has also been found desirable to mount the incubator on a nonmicrophonic support. This can be done conveniently by placing two dozen tennis balls in position on a base of sand and setting the incubator on top of them.

In first setting up this amplifier the values of the plate resistors must be adjusted to conform to the particular tubes used. The desired values are those at which the tubes are so biased that they take no grid current (that is, the grids are not positive) and that they have a normal plate current flowing (that is, the grids are not too negative).

These conditions can be determined by temporarily putting meters at the points marked "*x*" on the diagram. Meters having a range of 0-1.5 milliamperes D. C. should be put in the grid circuits (the points marked *a*, *b* and *c*). Meters having a range of 0-10 milliamperes D. C. should be put in the plate circuits (at points marked *d*, *e* and *f*). With these meters reading the currents in their respective circuits, the plate resistors can then be adjusted (starting with the one in the plate circuit of the first tube) until all grid meters read zero and all plate meters read from 1.5 to 2.5 milliamperes. The approximate values for the resistors with average tubes is between 12,000 and 15,000 ohms.

Once set these values will not have to be changed if the "B" battery voltages are maintained at their original values. If the tubes are changed the resistors must of course be readjusted.

The D. C. plate current in the last tube must be balanced out by a bucking battery and resistor so that the string galvanometer will not record a constant D. C. potential when the condensers are not in the circuit. This can be accomplished in the manner shown in Fig. 2. A high voltage and a large resistor should be used in order to keep the combined resistance in the plate circuit at somewhere the magnitude of the internal tube resistance and so gain the maximum output from the tube. The resistor used should be of high quality and capable of maintaining a constant value. The battery and resistor used in this installation were 45 volts and 0 to 25,000 ohms respectively.

In spite of the utmost care in construction there is likely to be a slow drift of the galvanometer due to D. C. variations somewhere in the amplifier. For satisfactory work this must be filtered out. The best method of accomplishing this is to put a large condenser in series with the string galvanometer. This filters out the D. C. component and gives a quiet string. The objection to this method is the distortion it produces in the wave form, particularly in a slow wave such as the T-wave. This distortion can be reduced to an inappreciable amount by using a condenser of about 50 microfarads. This condenser must be free from leakage in order to keep all D. C. drift out of the string. The condenser used on the present amplifier was built up of 2 microfarad paper condensers and has been quite satisfactory.

A good shunt must be used on the galvanometer to protect it when adjusting the apparatus. Extreme care should be taken in opening this shunt, since in spite of all precautions a current may be flowing through the string circuit. This shunt should have a very positive short circuit contact and should have very small steps as the shunt is first opened. The shunt built for this installation has the following steps: short, 0.05, 0.05, 0.05, 0.1, 0.1, 0.1, 1.0, 1.0, 1.0, 10, 50, 200, 800, 2000 and 4000 ohms.

The string galvanometer must also be chosen with care for this work. Because of leakage above ground in some types of amplifiers it was found that the Sanborn Electrocardiograph String Galvanometer would stand the strain excellently. The string in this circuit is not grounded and hence will not be sensitive to leakage currents. Another type of galvanometer was used at first but the string breakage was excessive.

The amplifier as described has been used successfully in taking a series of electrocardiographs of chicken embryo hearts immersed in a saline medium. A new amplifier using special nonmicrophonic tubes of the so-called airplane type and having more complete shielding is under construction.

We are indebted to the Willard Storage Battery Company for the loan of the storage batteries used in this apparatus.

REFERENCE

1. Pollack, H.: Electrocardiographic Studies on Chick Embryo Hearts. I. A Technic for Recording Electrical Changes in Isolated Chick Embryo Hearts, Jour. Lab. & Clin. Med. **16**: 1194, 1931.

LABORATORY METHODS

MECHANISM OF THE MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS*

PRELIMINARY REPORT

BY B. S. KLINE, M.D., CLEVELAND, OHIO

MICROSCOPIC slide precipitation test emulsions contain microscopically visible lipid (antigen) coated cholesterin plates which clump after being acted upon by positive syphilitic serum or spinal fluid. There is probably a combination of particle and reacting substance and a consequent surface alteration with lessening of solubility. The reaction in reality is one of agglutination rather than of precipitation.

Emulsions.—Emulsions generally employed in precipitation tests for syphilis are made by mixing salt solution with cholesterinized alcohol. The resultant aggregates are composed of antigen lipid, adventitious substances and cholesterin uniformly distributed throughout the particles. The size and shape of these units are determined especially by the quantitative relationship of lipoid and water, by the temperature of the ingredients and by the speed at which the mixture is made. In emulsions chemically identical for instance, the particles vary from less than 1 micron in diameter when very warm ingredients are quickly mixed, to large needle-like particles 60 micra or more in length when very cold ingredients are slowly mixed. The sensitivity of such emulsions, depending in part upon the size and shape of the antigen particles, is unavoidably variable unless strict precautions relating particularly to the quality and quantity of the antigen, quantity of salt solution, temperature of ingredients, speed of mixture and size of mixing vials are taken.

In contrast to such emulsions, those for the microscopic slide precipitation tests for syphilis are made by first precipitating the cholesterin crystals in water and subsequently coating them with antigen lipid. Cholesterin crystals precipitated from alcoholic solution by a small or large amount of warm or cold water or salt solution by slow or rapid mixture vary but little in size and shape. Upon this fact depends the greater uniformity in particle type of slide test emulsions. Because of it and the fact that the reacting surface of the final particle is composed of antigen lipid only and not of both antigen lipid and cholesterin there is a greater specificity and a greater uniformity of sensitivity of slide test emulsions in routine practice.

*From the Laboratory Department, Mount Sinai Hospital of Cleveland.

The clumps in positive Sachs-Georgi and Meinicke tests were found by Niederhoff[1] to be composed of the alcohol and ether soluble substances of the antigen extract and but traces of protein. Likewise, the clumps in positive microscopic slide precipitation tests washed a few times with distilled water consist in great part of substances giving the chemical reactions of cholesterin and lecithin and traces only of protein.

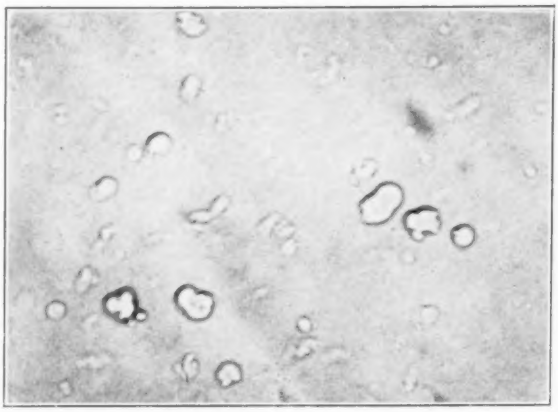


Fig. 1.—(Photomic. high mag.) Discrete globules of emulsion 1 composed of antigen lipid only in normal serum (negative reaction).

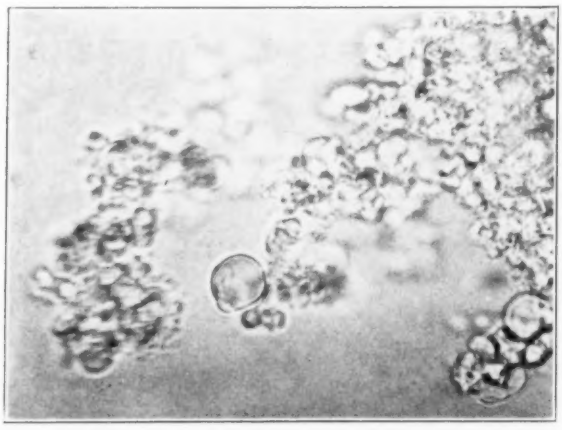


Fig. 2.—(Photomic. high mag.) Clumped globules of emulsion 1 in positive syphilitic serum (positive reaction).

Examined through the microscope, the clumps of positive microscopic slide precipitation tests are observed to be composed of the coated plates of the emulsion. Furthermore, the morphology of the clumps in positive reactions may be changed at will since by varying the method of preparation of the antigen emulsion, particles differing in size and shape result and these, acted upon by positive serum adhere to form the clumps.

Formula 1 below is that of an emulsion of small globules. When it is acted upon by positive syphilitic serum, the clumps observed microscopically are found to be composed of small globules. Formula 2 is that of an emulsion

of very small granules, much like those of diluted Kahn antigen dilution and when it is used, the clumps are composed of very small granules resembling those in positive Kahn tests. Formula 3 chemically identical with formula 2 is that of an emulsion of large needle-like particles much like those of Müller's Ballung antigen dilution and when it is mixed with positive serum the clumps are observed to be composed of large needle-like particles resembling those in posi-

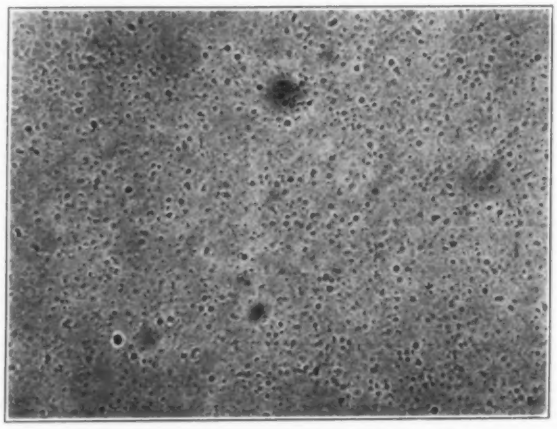


Fig. 3.—(Photomic. high mag.) Discrete very small granules of emulsion 2* composed of uniformly distributed antigen lipid and cholesterin in normal serum (negative reaction). (Resembling negative Kahn test.)

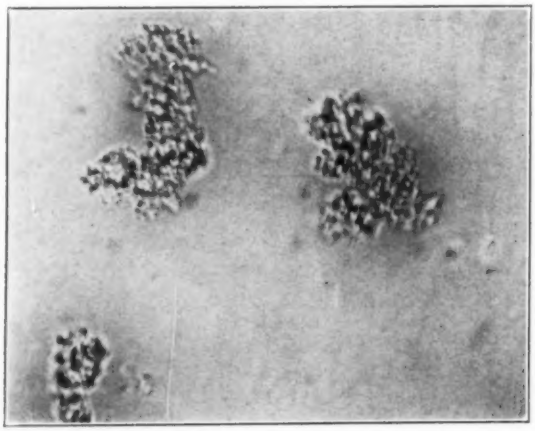


Fig. 4.—(Photomic. high mag.) Clumped granules of emulsion 2 in syphilitic serum (positive reaction). (Resembling positive Kahn test).

tive Müller's Ballung tests. Formula 4 is that of an emulsion of small plates as in the slide test emulsions and when it is employed the clumps are composed of small plates as in positive microscopic slide precipitation tests. (See Figs. 1 to 9.)

*Emulsions 2, 3 and 4 are chemically identical. The particle form of 2 and 3 is determined by proper quantity of water present, proper speed of mixture and proper temperature of ingredients. Strict precautions must be taken if these units are to be reproduced. The particle form of 4 is determined by the form of cholesterin crystals in water. These units are easily reproduced.

In slide tests, emulsions 1 and 2 are much less sensitive than emulsion 4. Negative, doubtful, positive and strongly positive reactions with all three emulsions are easily read with accuracy through the microscope at a magnification of about 120 times. The clumps in slide tests with emulsion 3 are less compact than those with emulsions 1, 2 and 4 but in tube tests (see below) the clumping is much more striking than with these emulsions and the results of negative, doubtful, positive and strongly positive reactions are easily read with the unaided eye. In the strongly positive tests, a single large ball results and the fluid clears as happens in Müller's Ballung test[2] in which similar large needle-like antigen units are employed.

Formula 1.—

(Ingredients at 21° C.)

1 c.c. slide test antigen to which is added slowly 1 c.c. distilled water or 2 c.c. of 0.85 per cent salt solution. Emulsion quite opaque yellow white. Small globules average about 6 micra in diameter.

Formula 2.—

(Ingredients at 21° C.)

0.1 c.c. slide test antigen.

1.25 c.c. of 1 per cent cholesterin in absolute (99+ per cent) ethyl alcohol in Kahn vial (a).

0.85 c.c. distilled water.

2.2 c.c. of 0.85 per cent sodium chloride in Kahn vial (b).

Slowly pour the contents of (b) into (a) and then back and forth quite rapidly 6 times.

Emulsion opalescent whitish. Contains very small granules average about 1 micron in diameter.

Formula 3.—

(Ingredients at 3 to 5° C.)

0.1 c.c. to 0.125 c.c. slide test antigen.

1.25 c.c. of 1 per cent cholesterin in absolute (99+ per cent) ethyl alcohol chilled in Kahn vial (a) placed in ice water (3° C.) one-half hour in refrigerator (8 to 12° C.).

1.75 c.c. of 0.85 per cent sodium chloride solution, chilled in Kahn vial (b) placed in ice water (3° C.) one-half hour in refrigerator (8 to 12° C.).

Rapidly pour contents of (b) into (a) and back and forth 4 times, allow to stand in ice water in refrigerator one-half hour.

Add 0.45 c.c. of 0.85 per cent salt solution and 0.85 c.c. distilled water to make emulsion chemically identical with emulsions 2 and 4.

Emulsion opaque whitish contains large needle-like plates average about 60 × 2 × 1 micra in diameter, scattered small globules and granules.

Formula 4.—

(Ingredients at 21° C.)

0.85 c.c. distilled water.

1.25 c.c. of 1 per cent cholesterin in absolute (99+ per cent) ethyl alcohol.

0.1 c.c. slide test antigen.

2.2 c.c. of 0.85 per cent sodium chloride solution.

Emulsion quite opaque whitish contains small plates average about 5 × 3 × 1 micra in diameter.

Sensitivity and Specificity.—In discussing the sensitivity of emulsions, Eagle[3] maintains "The coarsened dispersion state of antigen already shown to be caused by cholesterin completely explains its effect in increasing the sensitivity, i.e., the velocity of this aggregation." That the sensitivity of the antigen emulsion may be decidedly disproportional to its dispersion state, however,

is shown by the fact that results of tests with Kahn antigen dilution containing very small granules are no less sensitive than those of tests with Müller's Ballung antigen dilution composed of comparatively enormous particles. Furthermore emulsions with slide test antigen plates are strikingly more sensitive than those with solid antigen globules of larger size. The evidence indicates an optimal size and, just as important, an optimal form of particle for agglutination.

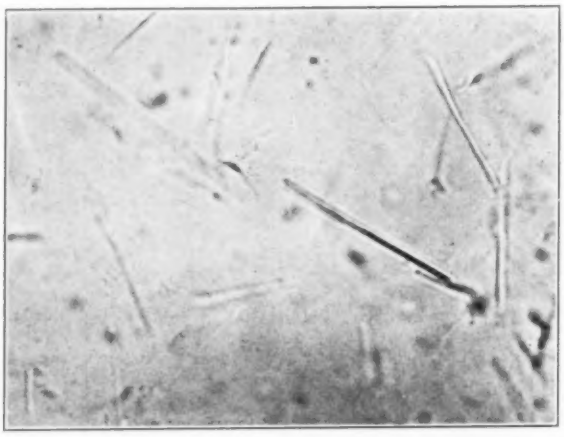


Fig. 5.—(Photomic, high mag.) Discrete large needles of emulsion 3 composed of uniformly distributed antigen lipid and cholesterin in normal serum (negative reaction). (Resembling negative Müller test.)

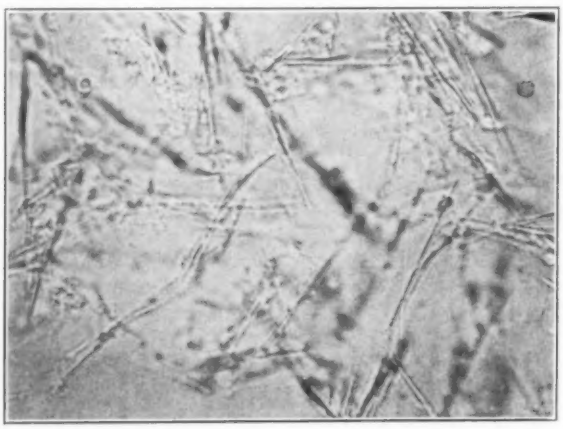


Fig. 6.—(Photomic, high mag.) Clumped needles of emulsion 3 in positive syphilitic serum (positive reaction). (Resembling positive Müller test.)

Furthermore, the sensitivity of an emulsion is affected by the quantity of antigen lipid, water, alcohol and electrolyte present and by the temperature to which the emulsion is exposed. Only when all other things in an emulsion are constant will coarser dispersion within limits determine greater sensitivity.

The dispersion state of the antigen units alone, however, determines the optimal method of reading test results. Whereas the reactions of the Müller test, and the ball test described below, both with large units (about 60 micra

in length) may be read accurately with the unaided eye, those of the microscopic slide test, the Meinicke Klarung test, the flocculation test proposed by Eagle[4] containing comparatively small units (averaging about 5 to 10 micra in diameter) and the Kahn test with very small units (about 1 micron in diameter) are best read through the microscope at a magnification of about 120 times.

Fig. 7.—(Photomic. high mag.) Cholesterin plates in water.

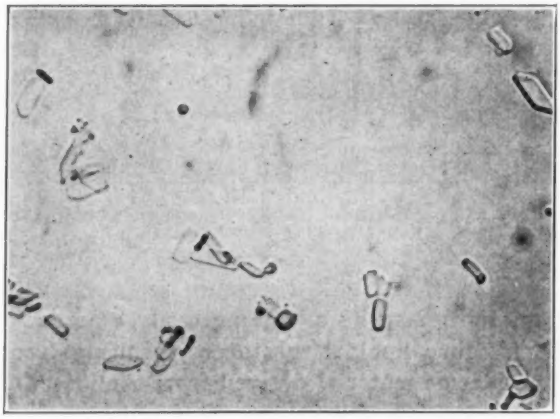


Fig. 8.—(Photomic. high mag.) Discrete antigen coated plates of emulsion 4 in normal serum (negative slide test reaction).

By proper preliminary treatment of the ingredients with cholesterin only as the sensitizer (as in Müller's Ballung emulsion and the ball test emulsion described below) an emulsion is obtained with much coarser particles than those of Eagle flocculation emulsions[5] employing cholesterol and sitosterol (in supersaturated solution) as the sensitizer.

The clumps of various positive flocculation reactions are microscopically indistinguishable from those due to the flocculation of the particles of the respective emulsions in a small quantity of water by alcohol (dehydration

effect) or by sufficient ordinary electrolytes (electrical neutralization effect or neutralization and dehydration effects) both of which effects produce no microscopically visible change in the antigen particles themselves.

Although not microscopically visible, there is evidence as presented by Eagle[6] that the mechanism of clumping of the antigen particles by syphilitic serum, is similar to that of specific bacterial agglutination, and due to a combination of altered globulin and antigen particle resulting in a surface with hydrophobic character.

The suggestion that this reacting substance may be an autoantibody to the products of tissue destruction in syphilis was first advanced by E. Weil.[7] Furthermore, since Landsteiner[8] raised the question of whether or not a lipoid haptene is liberated in the syphilitic process and if so whether by combination with the serum it induces the formation of the substance responsible for the positive Wassermann reaction, Sachs Klopstock and Weil[9] consider

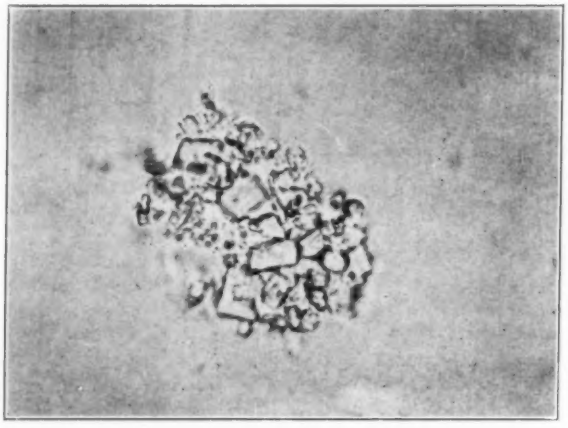


Fig. 9.—(Photomic. high mag.) Clumped plates of emulsion 4 in positive syphilitic serum (positive slide test reaction).

the evidence to indicate that the Wassermann reaction (an ultramicroscopically visible flocculation) may be a true antibody reaction of heterogenetic type in which homologous lipoidal substances resulting from tissue disintegration combine with some material derived from the infecting organism and form a complete antigen leading to autoantibody formation.

ANTIGEN EMULSIONS FOR THE MICROSCOPIC SLIDE PRECIPITATION TESTS FOR SYPHILIS[10]

Rôle of the Various Ingredients.—The antigen emulsions for microscopic slide precipitation tests for syphilis contain as do most antigen preparations for the diagnosis of syphilis the following substances:

Antigen lipid, cholesterin, water, sodium chloride, and ethyl alcohol. Each of these substances plays an important rôle in the emulsion.

(a) *Antigen Lipid.*—This consists of a waxy, brown substance, giving the chemical reactions of lecithin, obtained by precipitation in acetone at 50° C. to

37° C. of concentrated absolute alcohol extract of beef heart muscle powder (Difco).

Antigen lipid wax emulsifies in water in the form of globules. The more water present the smaller the resultant globules. With sufficient water the dispersion is partially submicronic. In emulsions with a small quantity of water the lipid globules clump on the addition of alcohol (dehydration effect) and strikingly enough go into solution on the addition of more alcohol.

This behavior of the lipid apparently explains the phenomenon recorded by Sachs and Rondoni[11] that the average size of the lipid particles in an emulsion depends upon the rapidity of mixture of the alcoholic solution of lipoid and water. When water is added slowly, more of it may be bound by the alcohol and less available for dispersion of the lipoid. The more quickly the water is added the less opportunity there is for it to be held by alcohol and the more will be available for the dispersion of the lipoid.

The globules of antigen lipid in a small quantity of water or salt solution when added in optimal quantity to strongly positive syphilitic serum, undergo clumping. The resultant clumps are of light specific gravity since on centrifugation they go to the top of the fluid. It is of interest to note that a positive agglutination reaction may be obtained with an emulsion containing no cholesterin or salt and that the total positive electrolytic effect may be supplied by the tested serum. Emulsions of antigen lipid only, in minimal quantities of water or salt solution, however, are insufficiently sensitive for use in the serum diagnosis of syphilis and accordingly cholesterin is added to secure stronger reactions.

(b) *Cholesterin (Pfanstiehl c.p.).*—The sensitivity of the antigen emulsion is dependent more upon the cholesterin content than upon that of the antigen itself. Satisfactory emulsions may contain, in a total of 4.4 c.c., from 0.0375 c.c. to 0.15 c.c. of the antigen (400 per cent range). The cholesterin content, on the other hand, must be more carefully titrated since the more nearly it approaches the maximum dispersible in a given total, the more sensitive the emulsion becomes. Beyond this point the emulsions are unsatisfactory because of the presence of nondispersible clumped particles. Emulsions with 0.75 c.c. of 1 per cent cholesterin in a 4.4 c.c. total give results of moderate sensitivity whereas those with 1.25 c.c. (60 per cent difference) of 1 per cent cholesterin in the same total with the same amount of antigen give very sensitive results.

The mode of action of the cholesterin in the slide tests apparently is a physical one as was thought to be the case in the Wassermann reaction by Browning, Cruickshank and M'Kenzie[12] who first reported cholesterin's property of increasing the sensitivity of Wassermann test antigens.

In preparing the slide test emulsions, the addition of alcoholic solution of cholesterin to water results in the formation of numerous small flat cholesterin crystals in clumps. There is strikingly little variation in the size and the shape of the crystals when the alcoholic solution of cholesterin is mixed slowly or rapidly with little or much warm or cold water or salt solution. Upon this fact depends in considerable measure the uniformity in sensitivity of slide test emulsions prepared routinely according to a given formula.

After the cholesterin is precipitated, alcoholic solution of antigen is added resulting in the formation of solid antigen lipid globules the vast majority of which on vigorous shaking are quickly absorbed by the cholesterin plates, the lipid forming a surface coat about each plate. Finally salt solution is added and in place of clumps of hydrophobic cholesterin crystals, discrete hydrophilic lipid coated cholesterin plates compose all but a small percentage of the formed elements of the emulsion and act as ideal units for agglutination by minimal quantities of the reacting substance in syphilitic serum or of ordinary electrolytes. The more cholesterin solution within a certain range added to a given quantity of water, the more cholesterin plates result. The more of these plates, the more a given quantity of added antigen will be spread over units with optimal reacting surface and the less will emulsify in solid globular form and the more sensitive the emulsion will be.

It is conceivable, however, that cholesterin may increase the sensitivity of the slide test emulsion by affecting the solubility of the antigen absorbed on the surface of its crystals much as particles of collodion absorbing egg albumen on their surface render that substance less soluble in water as pointed out by Loeb.[13]

(c) *Water.*—In the presence of water, cholesterin in alcoholic solution precipitates in the form of flat plates in clumps. Variations in the speed of mixture and the quantitative relationships of the two fluids within a considerable range result in comparatively little difference in particle size contrasting markedly with the striking difference when water is mixed with alcoholic solution of both cholesterin and antigen as described.

Antigen lipid emulsifies in water in the form of globules and if, as in the preparation of slide test emulsions, cholesterin plates are present, the lipid globules spread over the surface of these.

The more water present at the time of the emulsification of a given quantity of antigen lipid the smaller the globules are.

(d) *Salt.*—Although antigen globules in a minimum quantity of water undergo clumping when acted upon by strongly positive serum, such emulsions give negative results with less strongly positive sera whereas these cause clumping of antigen globules in a minimum quantity of 0.85 per cent salt solution. The antigen coated cholesterin plates of the slide test emulsions are likewise more susceptible to agglutination in the presence of salt because of a reduction of their electronegative charge. If a sufficient number of positively charged sodium ions in concentrated salt solution are added to the emulsion they alone cause agglutination of the antigen particles, the clumps resembling those produced by the action of the reacting substance in syphilitic fluids. A similar clumping results when the emulsion is acted upon by negative serum to which a sufficient amount of salt has been added.

(e) *Alcohol.*—It was found that preparations with concentrated alcoholic solution of the (antigen) lipid are most satisfactory since they permit of the dispersion, in a given total, of more cholesterin particles than those prepared with the same quantity of the lipid in from five to ten times the quantity of alcohol. Furthermore, such emulsions are relatively stable, keeping (at room temperature) their antigenic properties undiminished for forty-eight hours.

Much more stable than these but less satisfactory for routine preparation are emulsions made from the wax itself (no alcoholic dilution). These (at room temperature) retain their antigenic power for weeks.

It has been found that to obtain emulsions of uniform sensitivity it is important not only that the chemicals contain no appreciable quantities of adventitious substances and be used in proper quantity but also that they be mixed in proper order. It is possible to secure uniformly satisfactory emulsions only when the cholesterin is precipitated before the antigen is added.

The following formula and method of mixture has been found thoroughly satisfactory in producing emulsions of uniform sensitivity in diagnostic heated serum tests for syphilis.

Formula.—

0.85 c.c. distilled water (P_H about 6).

1.0 c.c. of 1 per cent cholesterin (c.p. Pfanstiehl) in absolute 99+ per cent ethyl alcohol.

0.1 c.c. antigen.

2.45 c.c. of 0.85 per cent sodium chloride (c. p. or reagent, Merck) solution (P_H about 6).

The antigen emulsion is prepared as follows:

Into a one-ounce bottle the required amount of distilled water (P_H about 6) is pipetted.

The bottle is held at an angle and the 1 per cent cholesterin in absolute (99+ per cent) ethyl alcohol is allowed to run along the side of the neck of the bottle.

The bottle is gently rotated from the neck for twenty seconds.

The bottle is held at an angle again and the proper amount of antigen is pipetted against the side of the neck of the bottle from a finely graduated pipette.

The bottle is promptly stoppered with a cork and shaken vigorously (the fluid thrown from the bottom to cork and back) for one minute.

Lastly the 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken less vigorously than previously for one minute.

One c.c. of the above solution is placed in a narrow test tube (12 mm. inside diameter) in a water-bath at 35° C. for fifteen minutes, since this furnishes a better end point than allowing emulsions to stand at variable room temperatures a given time before use.

The emulsions when examined under the microscope at a magnification of about 120 times, show numerous very fine particles and few small globules but no clumps whatever.

Emulsions differing somewhat in formula from that given above have been found advisable for diagnostic and exclusion tests with whole blood and spinal fluid and for these, advantage is taken of the fact that the sensitivity of the antigen emulsion may be progressively increased by heat (to 63° C.) and that the sensitivity is likewise increased by centrifuging and dispersing the sediment composed of the larger particles of the emulsion in a proper smaller quantity of 0.85 per cent salt solution.

OPTIMAL MICROSCOPIC SLIDE PRECIPITATION TEST CONDITIONS

Optimal slide test conditions include the use of properly prepared antigen emulsions in optimal proportion with serum or spinal fluid. More or less antigen emulsion in the mixture results in weaker reactions. This optimal proportion is determined not so much by the volume of the emulsion as by the number of particles present. For the heated serum test, for instance, the op-

timal proportion is about one part of emulsion to seven parts of serum. For the spinal fluid test on the other hand, the proportion is about one part of emulsion to thirty parts of spinal fluid. The particle proportion in the two tests, however, is about the same since the emulsion is centrifuged and concentrated for the spinal fluid test.

Open slide chambers with optimal surface for the mixture of the ingredients of the microscopic slide precipitation tests are employed since the results of the reactions in them of syphilitic fluids are much more positive than when comparatively narrow test tubes are used. This advantage is especially useful in the spinal fluid test and by its utilization, chemical concentration of the globulin as in the Kahn test is not necessary.

The agglutination reactions on the slides are rapidly completed by properly agitating the test ingredients.

In slide tests with unheated serum, unheated defibrinated blood and spinal fluid an optimal quantity of electrolytes must be added to secure results of proper sensitivity and relatively little more or less than the optimal quantity changes the result appreciably. Heated serum, on the other hand, is much less affected by the addition of ordinary electrolytes. In testing unheated specimens, therefore, it is of the greatest importance to use distilled water of known P_H (about 6), in the preparation of the necessary antigen emulsions, salt solutions and acid solution. Fresh unheated positive defibrinated blood or unheated serum mixed as is heated serum with slide test emulsion, causes much less clumping. When, however, a proper quantity of ordinary electrolytes (3 per cent to 5 per cent sodium chloride solution) is added to the unheated specimen the result equals in sensitivity that of the heated serum test. Addition of sodium chloride solution to the spinal fluid, however, does not increase its agglutinating capacity and for this purpose the more powerful acetic acid (1 per cent) is employed. For greater accuracy and ease in reading the results, these are observed through the microscope at a magnification of about 120 times (16 mm. objective, ocular 12X).

ANTIGEN EMULSIONS OTHER THAN THOSE FOR THE SLIDE TESTS AND OTHER PRECIPITATION TESTS

In the course of the study of the influence of the order of mixture of the ingredients on the character of the emulsions, it was found that when, as for most precipitation and complement-fixation tests for syphilis, alcoholic solution of both antigen and cholesterin is added to salt solution, emulsions of variable sensitivity result and that coincidently depending upon slight variations of procedure, the emulsions contain various sized and shaped particles. For instance, an emulsion of very small granules results with formula 2 (see above) and an emulsion of very large needles results with formula 3, the formulas being identical chemically.

Furthermore the variable sensitivity of emulsions prepared by mixing salt solution with alcoholic solution of both antigen and cholesterin according to the same formula depends in part upon a variation in size and shape of resultant particles determined by unavoidable variation in temperature of

ingredients, speed of mixture and the quantitative relationships of the lipoids and water at the instant of mixture. Such variation in particle size and shape is practically negligible when the cholesterin alone in alcoholic solution is first precipitated in water, even when the variations in temperature of ingredients, speed of mixture and quantitative relationships of the cholesterin and water at the instant of mixture are much greater than in the method generally employed.

Emulsion particles prepared by mixing salt solution with alcoholic solution of both antigen and cholesterin undergo greater changes due to environmental conditions including changes determined by the fluids of the emulsion, the serum to be tested and the room temperature than do slide test emulsions. In the fluids alone there is for instance a decided change in morphology of the lipoid particles in the first minutes after both cholesterin and antigen in alcoholic solution are mixed with salt solution, intensified considerably by varying the temperature of the mixture. Usually within one to several hours such marked secondary changes occur that the suspensions are unsatisfactory for use. Then as regards behavior with serum to be tested and room temperature, it has been observed that one such antigen dilution (Kahn) at low room temperatures in mixtures with negative serum on open slides develops a granularity resembling a weakly positive reaction with syphilitic serum at higher room temperature, a phenomenon not occurring when slide test emulsions are used under similar circumstances. Furthermore, when emulsions are made by mixing salt solution with alcoholic solution of both antigen and cholesterin, the resultant particles are more susceptible to higher temperatures than are particles in slide test emulsions and undergo much greater swelling and distortion of shape. This undoubtedly depends upon the fact that cholesterin crystals swell much more in water and salt solution when heated than do antigen lipid particles. For instance, at room temperature and at 35° C., cholesterin plates in water are of small size and when progressively heated to well above 65° C. increase to many times this size (there is at the same time a decrease in the compactness of the clumps). (Cholesterin plates in water heated in a sealed ampule at 100° C. dissolve. When the ampule is removed from the boiling water and the temperature falls the cholesterin reprecipitates and the plates are not only many times the size of those in similar quantity of water at room temperature but they are almost discrete and apparently changed in character since they no longer hold lipid antigen on their surface.)

Lipid antigen globules in a small quantity of water, on the other hand, when correspondingly heated (to 65° C.) undergo much less swelling than cholesterin plates and, furthermore, after being heated at 100° C. in a sealed ampule, they are barely visible through the microscope.

The particles of the antigen preparations made by mixing salt solution with alcoholic solution of both antigen and cholesterin behave as if composed of cholesterin and antigen lipid uniformly distributed rather than that they are composed of a core of cholesterin and a surface of antigen lipid as maintained by Eagle.[3]

Since aggregates of cholesterin and antigen so combined are more subject to change than those, as in the slide test emulsions, with a cholesterin

plate core and a reactive surface coat of antigen lipid only it is not surprising that the results of routine tests with mixed antigen cholesterin units show greater variation in specificity and sensitivity than when cholesterin plates coated with antigen are employed. These observations further emphasize the advisibility in antigen preparation of removing with acetone the adventitious substances in alcoholic antigen extracts so that the reactive surfaces of the particles in antigen emulsions shall be composed as nearly as possible of antigen lipid only.

BALL TEST FOR SYPHILIS

As stated above, one advantage possessed by an emulsion of large needle-like combined antigen cholesterin units is the fact that results of negative, doubtful, positive, and strongly positive tests with it are easily read with the unaided eye.

If strict precautions are taken in emulsion preparation the following test may be of value in supplementing the microscopic slide precipitation tests for syphilis.

The materials for the ball test are the same as for the microscopic slide precipitation tests except that tubes 4 inches long with 11 mm. inside diameter are employed instead of slides and the emulsion is prepared as follows:

Place optimal quantity, usually 0.2 c.c. to 0.25 c.c., of antigen (the same as employed for the slide tests) in a Kahn vial (a). Add 2.5 c.c. of 1 per cent cholesterin (e. p. Pfanstiehl) in absolute (99+ per cent) ethyl alcohol.

Place 3.5 c.c. of 0.85 per cent sodium chloride solution in Kahn vial (b).

Place the 2 vials in ice water (about 3° C. made quickly by throwing ice and water from pan to pan) in the refrigerator (8° to 12° C.) for thirty minutes.

Rapidly pour the contents of vial (b) into those of vial (a) and then back and forth four times (nine changes in all) and allow the mixture to stand in the ice water in the refrigerator for thirty minutes.

The emulsion is now ready for use and, kept at room temperature is satisfactory for several hours. (Not more than six hours.) The emulsion when examined through the microscope during this period shows innumerable large needle-like particles and scattered small globules and granules.

Upon standing at room temperature for longer than six hours, the needle-like particles of the emulsion increase in size and clumps of small globules and granules appear. The emulsion in this state is no longer satisfactory for use.

BALL TEST

Place 0.5 c.c. of each serum (heated at 56° C. one-half hour) to be tested in a tube (4 inches long, 11 mm. inside diameter) in a copper rack.

After the sera are pipetted add to each tube 0.1 c.c. of the chilled ball test antigen emulsion.

With a few rotary motions of rack or tubes separately, distribute the emulsion throughout the sera (avoid vigorous agitation).

Place the rack in a water-bath at 37° C. for 15 minutes.

Place the tubes in large centrifuge cups and centrifuge at moderate speed (unloaded about 1,000 r. p. m. third setting of Rheostat of International Equipment Company Centrifuge, Size 1, type SB) for thirty minutes.

Remove the tubes very carefully (avoid jarring) and replace in the copper rack.

Read the results at once according to the degree of clumping. Negative test, no clumps, fluid very turbid. Doubtful test, small clumps, fluid turbid. Positive test, few large clumps, fluid slightly turbid. Strongly positive test, one large ball, fluid clear.

COMMENT

A comparison of ball and slide tests with the same antigen is being made and will be reported upon later. To prevent, as much as possible, variations in sensitivity in the ball tests, strict precautions relating to quantity and quality of ingredients, temperature of ingredients at time of mixture and subsequently, speed of mixture, size of vials and time of chilling ingredients, time of ripening of emulsion and time of use of the emulsion must be taken. The optimal quantity of antigen to use is determined as follows: Emulsions are prepared as described above with the following quantities of antigen: 0.175 c.c., 0.2 c.c., 0.225 c.c., 0.25 c.c., 0.275 c.c., 0.3 c.c. After preparation in ice chamber, allow emulsions to stand at room temperature one-half hour, then mix one drop emulsion with six drops salt solution and examine under microscope at a magnification of about 120 times. Emulsions satisfactory for use contain large needles and very few small globules and granules. Unsatisfactory emulsions contain in addition to needles, fairly numerous globules and granules, some of these clumped.

SUMMARY

Microscopic slide precipitation test emulsions contain microscopically visible lipid (antigen) coated cholesterin plates which clump after being acted upon by positive syphilitic serum or spinal fluid. There is probably a combination of particle and reacting substance and a consequent surface alteration with lessening of solubility. The reaction in reality is one of agglutination rather than of precipitation.

Emulsions generally employed in precipitation tests for syphilis are made by mixing salt solution with alcoholic solution of both antigen lipid and cholesterin. The resultant aggregates are composed of antigen lipid and cholesterin uniformly distributed throughout the particles and are very responsive to environmental conditions, especially to the quantity of water present and the temperature of the ingredients at the instant of mixture. In emulsions chemically identical for instance, the particles vary from less than 1 micron in diameter when very warm ingredients are quickly mixed to large needle-like particles 60 micra or more in length when very cold ingredients are slowly mixed. The sensitivity of such emulsions, depending in part upon the size and shape of the antigen particles, is unavoidably variable unless strict precautions relating especially to temperature of ingredients, speed or mixture and size of mixing vials are taken.

In contrast to such emulsions, those for the microscopic slide precipitation tests for syphilis are made by first precipitating the cholesterin crystals in water and subsequently coating them with antigen lipid. Cholesterin crystals precipitated from alcoholic solution by a small or large amount of warm or cold water or salt solution by slow or rapid mixture vary but little in size and shape. Upon this fact depends the greater uniformity in particle type of

slide test emulsions and because of it and the fact that the reacting surface of the final particle is composed of antigen lipid only and not of both antigen lipid and cholesterin depends the greater specificity and the greater uniformity of sensitivity of slide test emulsions in routine practice.

The rôle of the various ingredients in the slide test emulsions and other factors influencing the sensitivity of the tests are discussed.

A description of a ball (tube) test for syphilis to supplement microscopic slide precipitation tests is given.

REFERENCES

1. Niederhoff, P.: Ueber die chemische Natur der bei der Sachs-Georgi und Meinicke-Reaktion, sowie bei dem Toxin-Antitoxin Nachweis nach Georgi auftretenden Flocken, München. med. Wchnschr. **68**: 330, 1921.
2. Müller, R.: Vereinfachte Methodik der Ballungreaktion (M. B. R. 11), Deutsche med. Wchnschr. **55**: 1624, 1929.
   Müller, R.: Über die vereinfachte Ballungs-Reaktion (M. B. R. 11) und ihre Beschleunigung durch die Zentrifugiermethodik, Med. Klin. **26**: 1193, 1930.
3. Eagle, H.: Studies in the Serology of Syphilis: III. Explanation of the Fortifying Effect of Cholesterin Upon the Antigen as Used in the Wassermann and Flocculation Tests, J. Exper. Med. **52**: 747, 1930.
4. Eagle, H.: Flocculation Test Proposed at Meeting of American Society of Clinical Pathologists, Philadelphia, Pa. (June), 1931.
5. Eagle, H.: Studies in the Serology of Syphilis: IV. A More Sensitive Antigen for Use in the Wassermann Reaction, J. Exper. Med. **53**: 605, 1931.
6. Eagle, H.: Studies in the Serology of Syphilis: I. The Mechanism of the Flocculation Reaction, J. Exper. Med. **52**: 717, 1930.
7. Weil, E.: Ueber den Lues-Antikörpernachweis im Blute von Luetischen, Wien. klin. Wchnschr. **20**: 527, 1907.
   Weil, E.: Das Problem der Serologie der Lues in der Darstellung Wassermann's, Berl. Klin. Wchnschr. **58**: 966, 1921.
8. Landsteiner, K.: Über heterogenetisches Antigen und Hapten. Mitteilung über Antigene, Biochem. Ztschr. **119**: 294, 1921.
9. Sachs, H., and Klopstock, A., and Weil, A. J.: Die Enstehung Der Syphilitischen Blutveraenderung, Deutsche med. Wchnschr. **51**: 589, 1925.
10. Kline, B. S., and Young, A. M.: A Microscopic Slide Precipitation Test for Syphilis (Second Communication), J. Lab. & Clin. Med. **12**: 477, 1927.
    Kline, B. S., and Levine, B.: 1000 Precipitation Tests for Syphilis With Small Quantities of Defibrinated Finger Blood (Clinical and Serologic Comparison), J. Lab. & Clin. Med. **15**: 768, 1930.
    Kline, B. S., and Littman, S.: Clinical and Serologic Comparison of the Microscopic Slide Precipitation Test for Syphilis and the Wassermann Test With the Same Antigen, J. Lab. & Clin. Med. **15**: 1008, 1930.
    Kline, B. S.: Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis, J. Lab. & Clin. Med. **16**: 186, 1930.
    Kline, B. S., and Rein, C. R.: The Microscopic Slide Precipitation Test for Syphilis With Spinal Fluid, J. Lab. & Clin. Med. **16**: 398, 1931.
    Kline, B. S.: Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis, Brit. J. Ven. Dis. **7**: 32, 1931.
11. Sachs, H., and Rondoni, P.: Beiträge zur Theorie und Praxis der Wassermannschen Syphilisreaktion. Ueber den Ersatz der Organextrakte bei der Reaktion, Ztschr. Immunitätsforsch, Originale. **1**: 132, 1909.
12. Browning, C. H., Cruickshank, J., and M'Kenzie, I.: Constituents in the Wassermann Syphilis Reaction, With Special Reference to Lecithin and Cholesterin, J. Path. & Bact. **14**: 484, 1910.
13. Loeb, J.: Stability of Suspensions of Solid Particles of Proteins and Protective Action of Colloids, J. Gen. Physiol. **5**: 479, 1923.

A MODIFICATION OF THE LANGE ACETONE TEST

By Eli Rantzman, Greensboro, N. C.

CLINICAL DIAGNOSIS by Todd, fifth edition, page 165, reads:

"Lange's Acetone test is a modification of the well-known Legal test. It is more sensitive and gives a sharper end-reaction. To a small quantity of urine add about one-twentieth its volume (1 drop for each c.c.) of glacial acetic acid and a few drops of fresh concentrated aqueous solution of sodium nitroprusside, and gently run a little ammonia upon its surface. If acetone be present, a reddish-purple ring will form within a few minutes at the junction of the two fluids. Lange's test is even more sensitive to diacetic acid than to acetone. For this reason, Rothera's test which is more sensitive to acetone is to be preferred: To 5 or 10 c.c. of urine, add about a gram of ammonium sulphate plus 2 or 3 drops of fresh concentrated sodium nitroprusside and overlay with strong ammonia. A reddish-purple ring shows the presence of acetone."

The desirability of a more permanent reagent would appear obvious. The modified reagent described below will keep at least six weeks before spoiling, is more economical and is far more sensitive than any of the reagents previously described, and yet so simple that it can be used in less than a minute's time. The only precaution necessary is to keep the reagent in a dark colored glass-stoppered bottle. Instead of making up a fresh solution of sodium nitroprusside each day, we have a solution that lasts several months. Our present solution was made two months ago and its sensitivity is described below.

Since little is known of sodium nitroprusside except its molecular formula, an investigation as to its stability would prove interesting. In the tests described as well as in the Legal test, specific mention is made that the nitroprusside solution used, must be freshly prepared, because sodium nitroprusside solution, when exposed to the air or light will lose its color within a few hours indicating the breaking down of the complex ion. However, should the solution be kept in an amber bottle, such decomposition would be prolonged if the bottle were corked. The ammonium nitrate plays no part in the reaction, except that it gives the reagent an acid reaction.

REAGENT

Keep Solution in an Amber Bottle.—Thirty-seven and five-tenths gm. of ammonium nitrate crystals, and 2.5 gm. of sodium nitroprusside crystals are dissolved in distilled water and the volume made up to 100 c.c.

PROCEDURE

Three c.c. of urine is placed in a test tube, to which is added 1 c.c. of reagent. Contents of tube are mixed and 2 c.c. of concentrated ammonia is

added by holding the test tube in an inclined position and allowing it to form a layer on top of the other without mixing.

REACTION

If the test is positive, a sharply defined purple or burgundy ring will appear at the point of contact. (To see the ring more plainly on pseudo cases, a white background helps considerably.)

The sensitivity of the reagent was shown by the following experiment. A series of six test tubes were set up. In the first tube 1.8 c.c. of distilled water was placed, while the remaining tubes received 1 c.c. Two-tenths c.c. of acetone was added to tube 1. The contents were well mixed by sucking the fluid up into the pipette and expelling it, then transferring 1 c.c. into the second tube, which in turn was well mixed and 1 c.c. transferred to tube 3. This was again mixed, transferred to tube 4, and continued until tube 6 was reached. One c.c. was then discarded from the last tube.

Two series of six tubes thus prepared were set up. Lange's Acetone test was done on one, while the New Method was done on the other. The results can be seen in Table I.

TABLE I

DILUTION OF ACETONE

	1-10	1-20	1-40	1-80	1-160	1-320
Lange's Test	++++	++++	+++	+	0	0
New Method	++++	++++	+++	++	+	+

The more dilute the acetone, the longer it takes the reaction to appear. In the first and second tubes of both series the color showed up almost immediately, while tube 3 took about one minute before reading could be made. Tube 4 took about two minutes, while tubes 5 and 6 took a little longer before readings could be made. Once the color appeared at zone of contact, it remained for more than twenty-five minutes before fading.

REMARKS

In 1922, my first experiment was made to find a substitute for the Lange test. In 1923, the present formula was obtained, and since then the formula has not been changed. The reagent has been used by myself while employed in The Good Samaritan Dispensary, Professional Pathological Laboratories, Prudential Life, and used exclusively for five years at the Jefferson Standard Life Insurance Company Laboratory.

It was but recently that the sensitivity of the reagent was discovered and because of that, these notes were written.

DOUBLE STRENGTH BROTH AND AGAR MEDIA*

By Pauline Epstein, New York, N. Y.

THE difficulty of obtaining adequate growth of streptococcus from various blood cultures is a well-known fact. Even with the very rich media, such as glucose phosphate, and potato phosphate, we frequently fail to recover the organisms in amount sufficient for complete and prolonged studies of various diseases caused by streptococcus. In addition, the successful growths on the previously used media are as a rule very short lived and any intensive study of the organisms was much complicated by the fact that the growth had to be continuously and repeatedly transplanted on new media. In our studies of rheumatic fever we were much handicapped by the absence of a favorable medium, which would remove these difficulties and after numerous experiments with media we found that the best results were obtained with the double strength broth which has been satisfactorily used over two years. Its composition as used in our laboratory and method of its preparation is as follows:

FORMULA

Procedure for Making Double Strength Broth:

1. Infusion for broth

Fresh lean chopped beef (bottom round) absolutely fat free	1000 gr.
Water, cold from tap	1000 c.c.

Infuse the meat overnight in ice box. Next morning boil the infusion until the meat clumps. Strain off meat through cheesecloth, squeezing out all the juice. Filter the juice through filter paper.

2. To this infusion add:

Peptone "difco"	1.0%	10 grams
Sodium chloride	0.5%	5 grams

3. Heat this mixture over the free flame to dissolve the ingredients.

4. Adjust the reaction to P_H 8.3 adding N/NaOH, 40-45 c.c. per liter. Add the NaOH while the broth is in the process of boiling.

5. After adjusting the reaction put the broth into the autoclave for ten or fifteen minutes under 15 pounds pressure to throw down the precipitate. Check reaction after autoclaving. The reaction should be P_H 8.0-8.1 to obtain final reaction of P_H 7.7. If the reaction is below this add more normal NaOH in order to counteract change during sterilization and boil the broth over the free flame five or ten minutes.

6. Filter through two funnels, the first one containing filter paper and cotton, the second one filter paper only. Through the first funnel (containing the filter paper and cotton) filter once. Through the second funnel filter several times until very clear.

7. Tube or bottle the broth and sterilize it in the autoclave at 15 pounds pressure for twenty or twenty-five minutes at a temperature of 249.9° F. Check the final reaction.

For three months blood cultures on rheumatic fever patients were taken using the single strength and double strength broth in parallel, and the results

*From the Department of Bacteriology, New York University Medical School.
Received for publication, January 23, 1931.

were, that on the double strength broth the growth appeared not only several days before the other kind, but also gave a considerably higher percentage of positive cultures.

During the first year we succeeded in recovering the organisms from the blood stream of 67 per cent of the rheumatic fever patients.

The second year, using the same broth, positive cultures were obtained in 80 per cent of the rheumatic fever patients.

However some batches of this broth are much better than others and occasionally the growth was scanty. The reason for such variations could not be explained otherwise than on the ground of the quality of the meat or change in the hydrogen-ion concentration. But as a rule, this broth has proved to be a good medium for enrichment. With the addition of any kind of blood or blood serum, ascitic fluid, or glucose, abundant growth is obtained. The organisms grown in this broth remain alive without transfer for at least six weeks, thus making an excellent medium for the preparation of stock cultures.

In the work with this medium, it has also been found that it produces a larger amount of hemolysins with pneumococcus, Streptococcus vividans, Streptococcus hemolyticus and the rheumatic strains.

From all these experiments we therefore come to the following conclusions:

1. Double strength broth is the most suitable medium in our possession at present.
2. It is a useful medium for the preservation of stock cultures.
3. It is a good medium for enrichment.
4. It is more favorable for the production of hemolysins.

These results were also confirmed by the double strength agar.

FORMULA

Double Strength Agar:

1. For double strength agar use the same procedure for the preparation of the infusion. Add to the infusion:

Peptone	1.0 per cent
Sodium chloride	0.5 per cent
Agar, chopped shreds	1.5 per cent
Normal NaOH as above.	

2. Put in the autoclave at 15 pounds pressure for thirty minutes to dissolve the ingredients.

3. Check the reaction and boil and filter through cotton until very clear.

4. Finish as above for double strength broth.

La Motte Indicator Set and corresponding Phenol Red Indicator (P_H 6.8-8.4) prepared by them was used to check titrations.

A METHOD FOR URINARY UREA*

Modified Folin and Youngburg

By Miles J. Breuer, M.D., Lincoln, Neb.

A SEARCH was made of the methods of determining urinary urea for the purpose of finding a method which was accurate enough and convenient enough to be used in clinical laboratories in office and hospital, for the purpose of determining kidney function according to Van Slyke's† "Urea Clearance" formula, $U/B\sqrt{v}$, where U is the urinary urea, B the blood urea, and v the number of c.c. of urine passed per minute over a period of an hour. Van Slyke states that the normal value of this function is 54 and the maximum 75.

The Doremus method of measuring nitrogen liberated by means of sodium hypogromite is notoriously inaccurate, both because of the neglect of factors of temperature and pressure in the nitrogen volume, and because other nitrogenous substances are decomposed into nitrogen. Marshall's method of titrating one specimen which has been treated with urease and another one not so treated, and deducting the difference, which is largely due to urinary ammonia, is fairly quick and convenient in a laboratory where this test is done as a constant routine. Where it is done only occasionally, the difficulty of preparing and the uncertainty of maintaining standard acid solutions for the titration puts the method into the second best place.

Folin and Youngburg's method involving the removal of urinary ammonia by permutite and the nesslerization of the urea nitrogen, offers the most promise, but is rather cumbersome as originally presented, and open to a number of objections from a practical clinical standpoint. The first one is that the large quantities of water necessary for dilution contain the possibility of introducing an error due to laboratory ammonia; and when the effort is made to free the distilled water from ammonia as suggested in the method, it is difficult, in such large quantities, to free it completely from permutite, which introduces errors into colorimeter matching. The large amount of unknown solution recommended is wasteful of Nessler's solution. The complicated steps of dilution increase the complexity of the calculation.

A rearrangement of the steps of the process has been found possible, resulting in a simplification of the dilution process and of the calculation, and has made it possible to use such a small total quantity of distilled water that it can readily be cleared of permutite, if necessary, by centrifugation. However, a checking up of the factor of the ammonia content of the distilled water has convinced me that if the water is properly distilled and preserved, its ammonia content need cause no concern, and no steps need be taken to remove ammonia from it.

*Received for publication, March 6, 1931.

†Van Slyke, et al.: Studies of Bright's Disease, Williams Wilkins, 1930.

If the still is run so that the condenser water flows hot enough to steam visibly, and if the distilled water is kept stoppered, after two weeks it does not give enough color with Nessler's solution to be distinguishable in the colorimeter. Duplicate determinations run with ordinary distilled water, and with distilled water freed from ammonia by permutite, show a difference of less than 1 per cent, which is within the limits of experimental technic. The following modification of Folin and Youngburg's technic is recommended for clinical use:

1. Dilute the urine 1:40 (if specific gravity is very low, dilute 1:20). Put about 2 gm. of permutite into a centrifuge tube, fill with the diluted urine, shake well, and centrifuge until clear. This is for the purpose of freeing the diluted urine of ammonia.

2. Put 2 c.c. of the clear, supernatant liquid into a 15 c.c. graduated centrifuge tube, together with one urease tablet dissolved in 1 c.c. of water, and add one drop of pyrophosphate solution.

3. Put the tube into a beaker of water at 50° C. and keep at that temperature for fifteen minutes.

4. Make up to 10 c.c. Centrifuge until clear. Take 5 c.c. of the clear fluid, dilute to 20 c.c., add Nessler's solution to maximum color, which is about 5 c.c. Dilute still further if necessary to obtain a color that is workable in the colorimeter.

CALCULATION

In this laboratory, where the Klett colorimeter with permanent glass standard is used, the calculation is as follows: Multiply the colorimeter reading by the glass-correction, 0.95. This gives R.

$$15/R \times \text{dilution} \times 40 = \text{mg. of urea nitrogen per 100 c.c.}$$

The "dilution" means the total volume of Nesslerized fluid.

If the original dilution is 1:20, substitute 20 for 40 in the equation.

Where the Duboscq type of colorimeter is used, make up a standard nitrogen solution containing 1 mg. of nitrogen per 5 c.c. of standard. Nesslerize 5 c.c. of the standard solution as near as possible simultaneously with the unknown, and dilute to 100 c.c. If the plunger on the standard side is set at 15, the same calculation will then apply, except that the glass-correction factor, 0.95 is omitted. If it is desired to set the plunger on the standard side at any other figure than 15, the following equation will apply:

$$S/U \times \text{dilution} \times 40 = \text{mg. of urea nitrogen per 100 c.c.}$$

Urinary urea readings are customarily reported in grams per c.c. In the Van Slyke formula they should be retained in mg. per 100 c.c., in order to be in the same terms as is the blood urea. If it is desired to change the above reading to grams per c.c., divide by 100,000, or move the decimal point five places to the left.

In the Van Slyke formula, it is necessary to express the result as urea nitrogen. If for some reason it is desirable to change the reading into urea, multiply by 2.14.

A DOG MUZZLE DESIGNED TO PREVENT COPROPHAGY*

By J. W. Spies, New Haven, Conn.

DURING the course of experiments necessitating a strict dietary régime which was deficient in vitamin D principle, it was observed that the dogs were eating their feces.[1] The prevention of the coprophagy with the least possible discomfort to the dogs was secured by the employment of a special muzzle (see Fig. 1). This device has proved so satisfactory that it is hoped that a duplicate, or a modification, may serve the purpose of other workers who wish to effectively combat coprophagous habits developing in their animals. The framework of the muzzle is of light, galvanized wire netting (3 meshes to the inch) which is soldered on the upper side to a longitudinal strip of tin, the

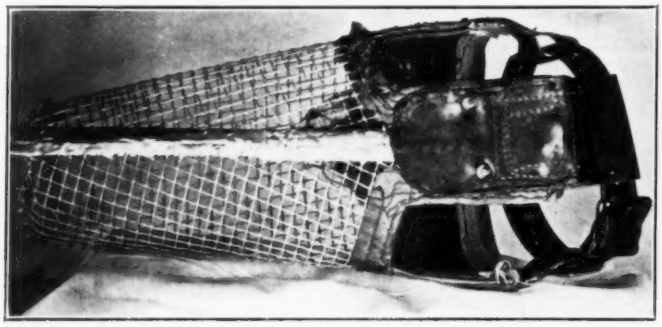


Fig. 1.—The explanation of the figure is given in the text. The muzzle from which this photograph was taken had been in constant use for four months.

latter being folded once upon itself. The general shape is that of a cone with the smaller end (which is closed) projecting well beyond the nose of the animal. The upper and posterior part has two large apertures which admit the ears. Four pieces of leather are fastened to the posterior end of the muzzle, and through the loops formed by these passes a leather strap which is buckled about the neck of the dog. Points of contact, which might cause irritation, are padded on the inside by felt, the borders being faced with cloth, and the whole then stitched to pieces of leather covering corresponding outside parts of the muzzle.

It is more satisfactory to fit each individual animal, but a muzzle slightly too large may be used quite well, since the leather strap encircling the neck may be adjusted with ease. Care should be taken to have the vision of the dog unobstructed from the front and sides. It is important that ample space be provided for unhindered panting.

It is necessary to remove the muzzle for feedings, but with a suitable

*From the Department of Surgery Yale University School of Medicine.
Received for publication, April 24, 1931.

water container of sufficient depth, drinking may be easily accomplished with the muzzle in place.

REFERENCE

1. Spies, J. W., Wilson, R. H., and Stringham, J. A.: Action of Viosterol and Parathormone in Thyroparathyroideetomized Dogs, Proc. Soc. Exper. Biol. & Med. **28**: 527, 1931.

RETICULOCYTE COUNT PREPARATIONS: AN IMPROVED TECHNIC*

By Jerome E. Cook, M.D., Herman M. Meyer, M.D., and Louis L. Tureen, M.D., St. Louis, Mo.

THE work of the past few years in connection with pernicious anemia has given added importance to the estimation of the reticulated cells of the blood. Any modification of the technic of such estimations in the direction of accuracy, simplicity or timesaving should therefore be welcome. No claim of originality is made for the method to be described. It is an adaptation of principles already in use in one form or another.

Reagents: A. A 1 per cent solution of brilliant cresyl blue in physiologic salt solution. The use of a little heat in dissolving the cresyl blue as well as the subsequent filtration of the cooled solution seems advantageous.

B. A 1 per cent solution of potassium oxalate in physiologic salt solution.

Preparatory to use one drop of B is added to five drops of A in a small clean test tube or watch glass. A small drop of blood is drawn into a capillary pipette of the sort used in the Sahli Hemometer. An equal amount of the oxalated stain is then drawn into the pipette and the blood and stain thoroughly mixed by alternately blowing them out onto a glass slide (one with a concavity, such as is used for hanging drop preparations is particularly well suited) and redrawing into the pipette several times avoiding air bubbles. A drop of the mixture is then placed near one end of a clean slide and spread in the usual manner for making blood smears. Especial care to dry the spread quickly is necessary to prevent distortion of the cells and a thin spread will be found advantageous. After drying, the spread is counterstained lightly with Wright's stain. Counterstaining gives good contrast and facilitates counting, but is not indispensable. A ruled ocular or one fitted with a small diaphragm likewise facilitates the count.

We have found this technic to compare well in accuracy with other methods and to have certain advantages. It requires no special apparatus such as centrifuge, special pipettes, etc. The smears can readily be made at the bedside and are rather uniformly satisfactory even in the hands of a novice. The slides are quite free from precipitated stain, which makes for accuracy in counting. The preparation of the smear takes scarcely more time than the making of the routine blood smear and can be utilized for the purposes of such a smear as well as for the reticulocyte count. Semipermanent preparations can be made by mounting in balsam.

*From the Medical Service of the Jewish Hospital of St. Louis.
Received for publication, April 24, 1931.

A SIMPLE AND ACCURATE METHOD FOR THE DETERMINATION OF CHOLESTEROL IN BLOOD SERUM OR PLASMA*

By Adolph Bernhard and I. J. Drekter, New York City, N. Y.

THE method for the determination of cholesterol in blood serum published by one of us[1] in 1918 has been used as a routine procedure in this laboratory since that time and was found to give very consistent results. In the course of certain investigations it became necessary to devise a method for cholesterol determinations, which would give as accurate results as the above procedure, but which would be simpler in manipulation, and require less time and material. The method which was developed and which is recorded in this paper is based upon the mechanical details of a method published by Harnes[2] for the determination of lipoid phosphorus.

Reagents—Alcohol-ether. Equal parts of 95 per cent ethyl alcohol and pure ethyl ether.

Redistilled acetic anhydride and chloroform.

Standard cholesterol solutions. A. Dissolve 150 mg. of pure cholesterol in redistilled chloroform, and make up to 250 ml. in a volumetric flask. Five c.c. of this solution contains 3 mg. of cholesterol.

Solution B. Dilute 5 c.c. of solution A, with 5 c.c. of chloroform. Five c.c. of this solution contains 1.5 mg. of cholesterol.

Solution C. Dilute 5 c.c. of solution B with 5 c.c. of chloroform. Five c.c. of this solution contains 0.75 mg. of cholesterol. These standards cover a wide range of cholesterol values.

Technic of the method. A strip of fat-free filter paper (Whatman No. 100 fat-free for milk analysis) is cut in half lengthwise. A strip about 160 mm. in length is impregnated with one c.c. of serum or plasma which is spread out in such a manner as to occupy about 7/8 of the length of the paper. The paper with the serum or plasma is air dried for fifteen to thirty minutes. When dry it is folded accordion-like, and placed in a Folin sugar tube, Fig. 1 (*A*) containing four c.c. of the alcohol-ether mixture. The paper should fit in the tube just above the constricted portion of the tube (*B*). A condenser, Fig. 2, consisting of a test tube 5 × 5/8″ fitted with a two-hole stopper, through one hole of which a long glass tube which reaches to the bottom of the test tube is placed, while in the other hole a short glass tube is fitted. This condenser (*C*, Fig. 1) is held in place on top of the Folin tube by a cork (Fig. 3) which has a slit or vent. The long glass tube is connected with the water intake. The Folin tube is placed in a slow boiling water-bath, and the alcohol-ether allowed to reflux for at least thirty minutes. The condenser and filter paper are removed at the end of this period, and the alcohol-ether is allowed to evaporate to dryness. The thoroughly dried and cool sediment of cholesterol is dissolved

*From the Achelis Laboratory and the A. Jacobi Division for Children, Service of Dr. J. S. Leopold, Lenox Hill Hospital.

Received for publication, April 25, 1931.

in 5 c.c. of chloroform, the contents of the tube agitated by tapping the bottom of the tube, and then two c.c. of acetic anhydride, and four drops of concentrated sulphuric acid (c.p.) are added. The contents of the tube are mixed by inversion and allowed to stand in a dark place for ten minutes; after which time comparison is made in the Klett or Duboseq colorimeter with the suitable standard solution of cholesterol in which the color has been developed as in the unknown.

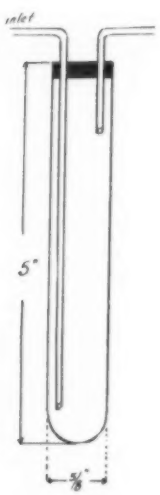


Fig. 2

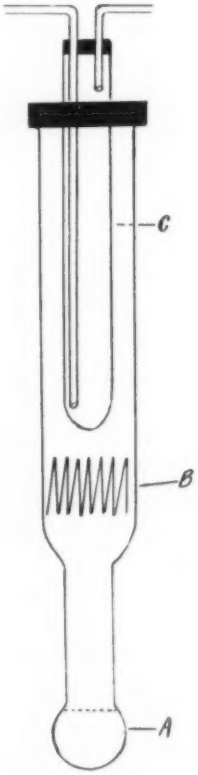


Fig 1

Fig 3

Calculation: $\frac{S}{R} \times p \times 100 =$ mg. of cholesterol in 100 c.c. of serum or plasma.

S Reading of the Standard.

R Reading of the Unknown.

p Strength of the Standard.

For the microdetermination, blood is obtained by puncture of the finger, and collected in a small centrifuge tube, allowed to clot and then centrifuged; two-tenths c.c. of serum are spread on the fat-free filter paper (35 × 100 mm.), dried and extracted as described above. The dried sediment of cholesterol is taken up in two c.c. of chloroform, and then one c.c. of acetic anhydride, and two drops of concentrated sulphuric acid are added, the tube is shaken and al-

lowed to stand in the dark for five minutes, and then compared in the colorimeter with two c.c. of the standard solution of cholesterol containing 0.3 mg. (prepared by diluting one c.c. of standard A with three c.c. of chloroform). The same amounts of acetic anhydride and sulphuric acid are added as with the unknown, the tube is then placed in the dark for five minutes and then compared in the colorimeter.

The macro method described above was used to determine the cholesterol content of 250 specimens of blood serum. Each determination was checked by the method of Bernhard[1] using two c.c. of serum. The results obtained by the new method varied between 70 and 468 mg. per 100 c.c. serum, as compared with 70 and 452 mg. obtained by the older method. The deviation between the two methods was minus two, to plus 14 mg., the average error being plus 5 mg. per 100 c.c. for the method described in this paper. These results are consistent, and comparable. Excellent colors for comparison are obtained, and there is no loss of cholesterol during the manipulation, neither is special apparatus required.

For the purpose of further checking the accuracy of the method, observations were made to determine (a) the time necessary for the complete extraction of cholesterol from blood serum, (b) the efficiency of the solvent used, (c) comparison with other methods.

(a) Time necessary for complete extraction of cholesterol. Each of seven strips of the fat-free filter paper was impregnated with one c.c. of the same serum. They were air dried for one-half hour, and transferred to the Folin tubes, each of which contained four c.c. of the alcohol-ether mixture. In another set of seven strips similarly prepared 4 c.c. of chloroform was used as the solvent. After extraction the condenser and paper were removed, and the solvent evaporated. The dried and cool residue was taken up in five c.c. of chloroform and the color developed. The results of these observations are recorded in Table I. It will be noted that when filter paper impregnated with blood serum is extracted with alcohol-ether, complete extraction of cholesterol is obtained with thirty minutes refluxing. Using chloroform as the extracting medium the results are inconsistent, and the values obtained are much lower. With serum E the filter paper after extraction with chloroform was re-extracted with alcohol-ether, and 110 mg. of cholesterol per 100 c.c. of blood recovered. It is evident therefore that alcohol-ether is more efficient as a medium for extraction of cholesterol, than is chloroform, when working with blood serum absorbed on filter paper.

(b) As a further check of these observations, and as a comparison with the gravimetric digitonin procedure,[3] large amounts of the same serum (25 c.c.) were spread upon fat-free filter paper, allowed to dry, then extracted in a Soxhlet apparatus, with alcohol-ether for twenty-four hours, and the cholesterol determined by the digitonin method. The same serum was also used for cholesterol estimations by both the new method and the method described by Bernhard. With the digitonin method 275 mg. per 100 c.c. was obtained, as compared with 312 mg. by the new method, and 300 mg. using Bernhard's technic.

With the same serum, using the same amounts on filter paper extracted with chloroform, in the Soxhlet apparatus for twenty-four hours, the cholesterol

TABLE I

SERUM	TIME OF EXTRACTION	SOLVENT	CHOLESTEROL 1[a]	CHOLESTEROL 2[b]
			Mg. per 100 c.c.	
A	15 min.	Alcohol-Ether	200	206
	30 min.	Alcohol-Ether	214	
	45 min.	Alcohol-Ether	214	
	60 min.	Alcohol-Ether	214	
	90 min.	Alcohol-Ether	214	
	120 min.	Alcohol-Ether	214	
	150 min.	Alcohol-Ether	214	
A	15 min.	Chloroform	50	206
	30 min.	Chloroform	60	
	45 min.	Chloroform	65	
	60 min.	Chloroform	70	
	90 min.	Chloroform	70	
	120 min.	Chloroform	76	
	150 min.	Chloroform	82	
B	15 min.	Chloroform	200	263
	30 min.	Chloroform	200	
	45 min.	Chloroform	214	
	60 min.	Chloroform	224	
	90 min.	Chloroform	236	
	120 min.	Chloroform	236	
	150 min.	Chloroform	236	
B	15 min.	Alcohol-Ether	263	263
	30 min.	Alcohol-Ether	270	
	45 min.	Alcohol-Ether	270	
	60 min.	Alcohol-Ether	270	
	90 min.	Alcohol-Ether	270	
	120 min.	Alcohol-Ether	270	
	150 min.	Alcohol-Ether	270	
C	45 min.	Alcohol-Ether	308	300
	120 min.	Chloroform	162	
D	45 min.	Alcohol-Ether	180	172
	120 min.	Chloroform	110	
E	120 min.	Alcohol-Ether	187	180
	+120 min.	Chloroform	60	

1[a] Cholesterol 1 Method reported in this paper.
2[b] Cholesterol 2 Bernhard 1918 method.
+The filter paper after chloroform extraction, re-extracted with alcohol-ether, and 110 mg. per 100 c.c. recovered.

content by the digitonin method was 230 mg. per 100 c.c. while with the new method with chloroform as the extraction medium 162 mg. was obtained. The filter paper after chloroform extraction was again extracted with alcohol-ether in the Soxhlet for twenty-four hours, and 40 mg. of cholesterol was recovered.

These experiments were repeated using a different specimen of serum. Two ten c.c. portions of the serum was spread on fat filter paper and dried. One was extracted with alcohol-ether in the Soxhlet for forty-eight hours, and the other was extracted with chloroform in the Soxhlet for the same period of time. The cholesterol was also determined colorimetrically, using both the new method described in this paper and the older method of Bernhard. Both alcohol-ether and chloroform were used as solvents. With alcohol-ether Soxhlet extraction and the digitonin method 156 mg. per 100 c.c. was obtained, while with chloroform-Soxhlet extraction and digitonin precipitation the amount recovered was 110 mg. The paper after the chloroform extraction was then again extracted in the Soxhlet with alcohol-ether as a solvent for twenty-four hours and 38 mg. additional cholesterol recovered.

With alcohol-ether extraction for forty-five minutes, 180 mg. was obtained using the new method as compared with 172 mg. by the Bernhard method. Chloroform extraction for one and one-half hours with 1 c.c. of this serum absorbed on filter paper and following the new method proposed above, gave 105 mg.

In the entire series of experiments when chloroform was used as the extraction medium, it was found that lower values were consistently obtained when compared with alcohol-ether. We believe that the proof that chloroform does not completely extract cholesterol from blood plasma or serum with filter paper as the absorbent is ample. This is somewhat of a surprise since chloroform is an excellent solvent for pure cholesterol and several methods utilizing it as the sole extraction medium have been published.[4, 5] Allardyce[6] using filter paper and cotton-batting as absorbents and chloroform as the solvent, obtained practically the same results as those reported here. He checked his work against the plaster-of-Paris method of Myers and Wardell,[7] which method gave him the highest values for the recovery of cholesterol. Allardyce obtained inconsistent results with filter paper and cotton-batting as the absorbent, but he did not use any other solvent. With each of the two absorbents used by him the extraction was incomplete. From the observations reported here it appears that it is a question of the solvent used and not the absorbent. Complete extraction from filter paper of the cholesterol is obtained when alcohol-ether is used, with chloroform this is not the case.

Upon re-extraction of the filter paper with alcohol-ether extraction after the chloroform an amount of cholesterol is recovered which is the exact difference as it appears on single extraction between the two solvents. It has further been shown both by the digitonin precipitation method and colorimetric estimations, that alcohol-ether completely extracts cholesterol from filter paper, while chloroform does not.

CONCLUSIONS

1. A macro and micro method is described for the estimation of cholesterol in blood serum or plasma which is simple, accurate and rapid.

2. Alcohol-ether is the most efficient extracting agent for cholesterol when filter paper is used as the absorbent.

REFERENCES

1. Bernhard, A.: The Determination of Cholesterol in Blood Serum, J. Biol. Chem. **35**: 15, 1918.
2. Harnes, A. R.: The Colorimetric Determination of Lipoid Phosphorus in Blood, J. Biol. Chem. **77**: 405, 1928.
3. Mueller, J. H.: A Comparison of the Results Obtained by the Colorimetric and Gravimetric Determination of Cholesterol, J. Biol. Chem. **25**: 549, 1916.
4. Lieboff, S. L.: A Simplified Method for Cholesterol Determination in Blood, J. Biol. Chem. **61**: 177, 1924.
5. Ling, S. M.: The Determination of Cholesterol in Small Amounts of Blood, J. Biol. Chem. **76**: 361, 1928.
6. Allardyce, J.: The Determination of Cholesterol in Blood, Can. J. Res. **3**: 115, 1930.
7. Myers, V. C., and Wardell, E. L.: The Colorimetric Estimation of Cholesterol in Blood With a Note on the Estimation of Coprosterol in Feces, J. Biol. Chem. **36**: 147, 1918.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ERYTHROCYTES: Rapid Measurement of, Sauder, H. R., and Toomey, J. A. Am. J. Dis. Child. **41**: 823, 1931.

Preparation of the Blood Smear: A deep puncture wound which bleeds freely is made in the subject's ear or finger tip. The first drop of blood is wiped off with gauze, and a portion of the second drop smeared thinly over the surface of a meticulously cleaned glass slide. A suitable smear dries almost instantly, and there must be no delay from the time the drop of blood appears until it is smeared out. The smear is then examined under a microscope before being stained. If the cells are not evenly distributed or are obviously distorted, the specimen is discarded. All smears are made on a glass slide of the same kind and thickness.

Staining of the Smear: Wright's stain, suitably aged, is used as a staining agent. It is important that the same batch of staining fluid be used for successive smears in a given series so that all of the cells in the series will be subjected to a staining fluid of exactly the same concentration. The amount of stain and diluent for each smear is carefully controlled, and the time intervals of the various steps in the staining must be exactly the same in successive preparations. All smears are washed free of excess stain for the same period of time in distilled water. If the foregoing steps are observed, all smears of a given series will be uniformly fixed and stained.

Apparatus: The principle of the method involves the projection of the microscopic image of the cells to a suitable screen. The projection apparatus which is most ideally suited to this work is the Bausch and Lomb euscope. This instrument, by means of a prismatic reflector, projects the image of a microscope field to a verticle ground glass screen situated 50 cm. from the prism. This apparatus is rigidly constructed, and when set up the magnification factor does not change, although the apparatus may be accidentally subjected to a considerable jarring and bumping. Any good microscope with substage condenser can be adapted to this apparatus. The objective is a 1/12 Leitz chromatic oil immersion lens with a numeric aperture of 1.30. The ocular is a number 10 lens of the same kind. This combination of lenses with a tube length of 165 mm. gives a magnification factor of about 1,700 on the projection screen. If available, an apochromatic lens system would lend itself well to the apparatus, but it is not essential. A mechanical stage is necessary. The source of light is a 108 watt Mazda lamp equipped with an aspheric condenser lens. White light is used. The working distance of the light is 20 inches (50.8 cm.). The apparatus, of course, must be set up in a dark room. With this outfit, excellent definition of the cell images is obtained, and the boundaries of the cells can be readily and definitely located. The cell can be sharply focused. There is little trouble with the diffusion zone.

The Calibrated Rule: With the magnification of the apparatus adjusted to 1,700, a length of 1 micron projected on the screen measures 1.7 mm. In order to facilitate the estimation of the diameters of the cells, a special calibrated rule was devised. The smallest divisions on the rule measure 0.85 mm., which is equivalent to 0.5 micron in the diameter of a cell. In estimating the diameter of a cell, the tips of finely pointed, rigidly constructed screw calipers are superimposed on the extremes of this diameter. Then the calipers are placed over the rule, and the diameter read off directly in half microns.

The specially devised rule was made from an unexposed photographic film of suitable size. The emulsion was fixed and stained red with carbol-fuchsin solution. The calibration lines were scratched through the emulsion. The rule then presented clear lines on a red background, which offered good contrast to the lines and yet had a high degree of transparency. This transparent rule was then mounted directly on the ground glass screen of the euscope.

When the caliper points were placed on the rule, it was found difficult to hold one point on the zero line while the eye traveled to the other point to make the reading. To obviate this difficulty, the following scheme was resorted to: Two glass coverslips of suitable size with ground and polished edges were mounted in juxtaposition to the surface of the rule in such a manner that the line of apposition of the two cover glasses lay exactly over the zero line of the rule. When a reading was to be made, the calipers were moved over the surface of the rule until one point of the calipers engaged in the tiny fissure between the two cover glasses. It was then a simple matter to place the calipers in position rapidly and to make the reading.

Standardization of Apparatus: Standardization was accomplished by means of a standardized Leitz stage micrometer. The precaution to standardize the instrument each time to the same divisions of the micrometer was observed. The tube length of the microscope is so adjusted that the magnification factor was exactly 1,700. Frequent check-ups of this factor were made, but as the apparatus is very stable, no difficulty was experienced in keeping the magnification constant.

Measurement of Cells: By this method, all of the cells included in a small circular field outlined directly on the projection plate can be measured. The field is 6 cm. in diameter and is situated in the optical center of the apparatus. With the combination of lenses and condenser, all of the cells (from 18 to 25) included in such a field come into focus at the same point, and there is practically no difference in the diameter of cells situated in the center and on the periphery of the field.

The apparatus is now prepared to measure the cells. If a series of slides are being measured in which a change in the diameter of the cells is anticipated, the slides are given code numbers so that the operator does not know the sequence of the smears in the series. The slide is placed under the oil immersion lens, and about ten minutes are spent in the general inspection of the smear. In this way, a mental concept of the contents of the smear is formed, and if there happens to be any variation in the apparent size of the cell in various parts of the smear, this is noted. The observer is seated before the screen, and the adjustments of the stage are well within reach.

Having become thoroughly familiar with the contents of the smear, a suitable field is then chosen for measurement. The following are the criteria for such a field: (1) The cells must be evenly distributed over the surface of the field. (2) Fields should not be chosen in which there is obvious distortion of any of the cells. Ten fields are measured. If there are obvious differences in the average diameter of the cells of a field, attempt to measure an equal number of both extremes, the remainder of the fields chosen representing the median value.

With a suitable field chosen, proceed to measure the polar and equatorial diameters of the individual cells. Slightly or even moderately elongated cells are measured if the contour of the cell is regular and if the equatorial diameter represents the transverse diameter of the cell. The eye readily picks out the polar axis of a cell, and the points of the calipers are adjusted to the extremes of this axis. The calipers are then superimposed above the rule, and the measurement noted. In the same way, the equatorial diameter is determined.

The diameters are recorded on a suitable blank in groups of half microns. The measurements of the fields are kept on a separate record, so that the measurements of the field can be completed after the smear is completed. At least 200 cells are measured.

TUBERCULOSIS: Monocyte-Lymphocyte Ratio in Infants and Children, Hamil, B. M. Am. J. Dis. Child. **41:** 1023, 1931.

The supravital technic offers a simple, accurate method for the thorough laboratory study of the white blood cells in any disease.

Monocytes are increased above the normal figures in active tuberculosis. This observation is constant in severe tuberculosis, and the modified type of this cell predominates in severe and terminal stages of the disease. This type of monocyte is found in the blood of patients who do not have tuberculosis and is therefore not pathognomonic of the disease in infants and children.

The lymphocyte is the only type of cell in the circulating blood which appears to have a constant relation to the tuberculous process. The total lymphocyte count is decreased below the normal figure in all active types of tuberculosis, this decrease depending on the severity of the disease. The lymphocyte count is increased in the healing stage, the constancy of this increase depending on the progression of healing.

The total white blood cells and total neutrophils are increased in the terminal stage of severe tuberculosis. An increase in neutrophils is not constant in cases with ulceration and the formation of abscesses.

The ratio of monocytes to lymphocytes is increased in active tuberculosis, this increase depending on the severity of the disease. It is due to both an increase in total monocytes and a decrease in total lymphocytes, the latter factor being the more constant. As healing occurs, there is a decrease in the ratio, which is fluctuant until the healing is well advanced.

In cases with multiple lesions in different pathologic stages, no reliance can be placed on the monocyte-lymphocyte ratio to determine the status of the tuberculosis. The tuberculin skin test and treatment with ultraviolet light, in cases of chronic and advanced healing tuberculosis, cause a change in the monocyte-lymphocyte ratio, and blood studies should be interpreted in the light of such influencing factors.

It is suggested that fluctuations in the different types of white blood cells in the peripheral circulation of patients with tuberculosis are due to stimulation of the reticular cells to proliferation by products of the tubercle bacilli and the tuberculous lesions rather than either to the simple presence of tubercle bacilli in the body or to the demand for the different types of cells according to the type or stage of the pathologic lesion.

The great value of the white blood cell picture in tuberculosis in children lies in the distinction between active severe tuberculosis or an early healing tuberculosis and an advanced healing or latent tuberculosis in the child who shows some of the clinical signs of tuberculosis but no definite symptoms by which to determine the status of the pathologic process. Many children are brought to the practitioner for examination solely on the history of contact with tuberculous patients. They may show only a positive tuberculin test, which in children is a significant sign, and perhaps a positive D-Espine sign or a suggestion of hilar or suprahilar shadow beyond the normal limits. In such a case, the symptoms may be slight and in any event variable, although the pathologic process may be relatively extensive and rapidly progressive. A study of the white blood cells in such a case and a comparison of the total figures for each cell type with the normal total figures, and a computation of the monocyte-lymphocyte ratios will aid in determining whether the child should be permitted the freedom of home life, or whether he should be subjected to further observation under tuberculosis management.

There is no constant observation in the study of the white blood cells in the peripheral blood of children with tuberculosis that would warrant a diagnosis of tuberculosis without the support of clinical evidence of the disease.

By the use of the supravital technic, an accurate and thorough study can be made of the different types of white blood cells. However, since the total number of monocytes and the total number of lymphocytes are the significant factors in tuberculosis in children, a study of the cells by a fixed smear method of equal reliability, will serve to supply the information desired.

BLOOD: Chemical Detection of According to Segeloff, Schugt, P. Presse Wiss-prakt. Heft **166**: 7, 1930.

Reagents:

1. 0.5 per cent acetic acid.
2. 5 per cent solution of pyramidone in ether.
3. 3 per cent hydrogen peroxide.
4. 50 per cent acetic acid.

Urine is tested directly. Feces (30 gm.) must be triturated with 30 c.c. of 0.5 per cent acetic acid and filtered through a wet filter.

To 1 c.c. of urine (or feces filtrate) in a test tube add 8 drops of 50 per cent acetic acid, 8 drops of hydrogen peroxide, and then, with the tube inclined, add pyramidone reagent drop by drop.

The first 10 to 15 drops mix with the solution but on further addition two layers develop and, in the presence of blood, a light-blue to amethyst color at the zone of contact.

TULAREMIA: Rapid Agglutination Test, McDaniels, H. E. Am. J. Pub. Health 21: 552, 1931.

It has been found that adequate shaking of the tubes in the ordinary routine tularemia test enables a final report within about three minutes after setting up the test. A standard Kahn rack and tubes, or any test tube rack and tubes which will permit vigorous shaking, are suitable. The test is set up in the usual manner; it consists of a row of 8 tubes containing 0.5 c.c. each of serum dilutions ranging from 1:10 through 1:1,280 each succeeding tube having half the serum concentration of the preceding one. To each tube and a control containing 0.5 c.c. saline, 0.5 c.c. of formalin-killed antigen of a turbidity ordinarily used in macroscopic agglutination tests ($BaSO_4$ No.2—No. 4) is added. The rack is held in both hands and shaken for two minutes as in the Kahn test, and then gently for a minute longer.

BRUCELLA: Differentiation of, Huddieson, I. F. Am. J. Pub. Health 21: 491, 1931.

A total of 656 strains of Brucella from Europe and North America have been studied in their growth behavior toward aniline dyes in a suitable medium.

It has been found that these strains divide themselves into 3 groups or species according to the growth inhibiting action of thionin in a final dilution of 1-30,000, and pyronin in a final dilution of 1-200,000, in beef liver infusion agar at a P_H of 6.6.

Of the total number, 133 have been classified as Br. melitensis (Bruce), 352 as Br. abortus (Bang), and 172 as Br. suis (Traum). Br. melitensis grows on both the thionin and pyronin dye medium, while Br. abortus grows only on the one containing pyronin and Br. suis only on the one containing thionin.

The strains of Br. abortus may be divided into 2 classes; those which completely reduce basic fuchsin (final dilution 1-25,000 in beef liver infusion agar), and those which slightly reduce this dye. It appears, on the basis of isolated cultures, that the latter class is the one that is pathogenic for man.

When more than one species of Brucella occurs in blood culture or other infective material, the dye method and H_2S metabolism determination will separate and identify each of the species.

The growth inhibiting action of these dyes, especially pyronin, offers possibilities in the therapeusis of undulant fever in man and Brucella infections in animals.

FILMS: Preservation of Stained, Von Riemsdyke, M. Nederland Tijdschr. Hyg. Microbiol. en serol. 5: 119, 1931.

Stained smears may be preserved by painting them with a thin film of transparent gum arabic. ("Gee wee" gum 1 part; water 1.5-2 parts; make slightly alkaline to litmus with sodium hydroxide and sterilize.) The solution is quite stable but must always be alkaline.

The films must be dry.

NEUTROPENIA: Idiopathic, Baldridge, C. W., and Needles, R. J. Am. J. Med. Sc. 181: 533, 1931.

1. The name "agranulocytic angina" is unfortunate because of its intrinsic ambiguity, and because it implies a mouth infection which causes neutropenia instead of with a syndrome of utterly unknown etiology and pathogenesis.

2. Stomatitis is such a common secondary manifestation in the blood dyscrasias which are characterized by neutropenia that it is probably of no etiologic importance.

3. From the cases studied the authors concluded that neutropenia was primary and infection secondary, an idea which found final proof in the case reported by Roberts and Kracke.

4. In view of the inadequacy of present knowledge the authors feel that it is not justifiable to divide the syndrome arbitrarily, and suggest that all cases of unexplained neutropenia with secondary infection be grouped under the general head of idiopathic neutropenia.

5. The similarity between aplastic anemia, aleukemic leukemia and idiopathic neutropenia (especially the complicated forms) is emphasized.

6. The authors have included the record of a patient who was in the University Hospital in 1910 to illustrate the fact that in former years idiopathic neutropenia was either overlooked because the blood was not examined, or was interpreted as a symptom of bone-marrow exhaustion, aleukemic leukemia or aplastic anemia.

7. Splenectomy was performed in one of our cases with no apparent benefit.

8. The similarities which idiopathic neutropenia bears to anaphylaxis and the Shwartzman reaction are discussed.

ERYTHROCYTE: Methods and Clinical Value of the Determination of Size of Red Blood Cell, Haden, R. L. Am. J. Med. Sc. **181**: 597, 1931.

The following conclusions are advanced:

1. In a study of anemia it is important to determine the size of the erythrocytes; this may be expressed in terms of diameter or of volume.

2. By estimating the diameter of a sufficiently large number of red blood cells, the average diameter of a cell may be determined and the degree of anisocytosis may be measured.

3. The volume is a much more sensitive indicator of the variation in the size of red blood cells than is the diameter.

4. A 13 per cent increase in the diameter of an erythrocyte causes a 44 per cent increase in its volume.

5. The most accurate method of determining the cell mass is by the use of the hematocrit, using an isotonic anticoagulant, which does not change the volume of the cells.

6. It is best to express variations from the normal in terms of volume index.

7. The best fundamental classification of the types of anemia is based on the volume of the average blood cell.

8. Pernicious anemia always shows an increase in the volume of the cells; anemia due to a known cause usually shows a decrease in cell volume or a normal volume. An increased cell volume with achlorhydria is an almost absolute diagnosis of pernicious anemia.

9. The prognosis in an anemia due to known cause becomes progressively more unfavorable as the cell volume decreases.

10. The determination of the cell volume is the best guide in the treatment of anemia.

11. Iron therapy is probably of value only when the cells are below the point of hemoglobin saturation, as shown by a decreased saturation index.

FLAGELLA STAIN: Maneval, W. E. J. Bacteriol. **21**: 313, 1931.

Stain:

Water, distilled	30 c.c.
Basic fuchsin, 10 per cent alcoholic	10 c.c.
Anilin oil (1 part) and 95 per cent alcohol (3 parts) mixed	5 c.c.
Acetic Acid, 4 per cent	1 c.c.

Mix in the order given, filter once or twice and again before using. It is best after three to six days and may sometimes be kept for several weeks, but it finally deteriorates. Good results have been obtained by substituting rosanilin or methyl violet for the basic fuchsin in this formula. Anilin fuchsin and anilin gentian violet were poorer.

The author conducted a study of mordants, finding Nos. 1, 3, and 22 in the table to be useful for general use.

The preparation is practically the same for all of these mordants. The tannic acid, or else the tannic acid and silver sol mixed (Nos. 22-24), is heated to boiling in a Pyrex flask. The inorganic salt (Nos. 1-6 and 22-24), or the two inorganic salts mixed (Nos. 7-21) is added, a few drops at a time, as boiling continues. Then the alcoholic fuchsin is added and the mixture boiled two minutes longer. After cooling the mordant should be filtered (use Whatman No. 1 filters). Refiltering on successive days, just before using, is generally necessary.

These mordants are generally most satisfactory when one to four days old, with the exception of Nos. 22-24, which are best when a few hours or a day old. After two to five days they usually fail to work but may be "renewed" by bringing to the boiling point and adding 1 c.c. of inorganic salt or salts and 2 drops of alcoholic fuchsin per 10 c.c. of mordant. "Renewed" mordants are sometimes better than when first made. They should be filtered after standing a day and on succeeding days just before using.

FORMULAS FOR MORDANTS

MORDANT NUMBER	TANNIC ACID 10% C.C.	ALCOHOLIC FUCHSIN 10% DROPS	$AlCl_3$ 20% C.C.	$CuCl_2$ 20% C.C.	$FeCl_3$ 20% C.C.	$Fe(OH)_3$ SOL. C.C.	$NiCl_2$ 20% C.C.	$ZnCl_2$ 20% C.C.	SILVER S SOL. C.C.
1	20	10	4						
2	20	10		4					
3	20	10			2				
4	20	10				6.5			
5	20	10					4		
6	20	10						4	
7	20	10	2	2					
8	20	10	2		1				
9	20	10	2			3.5			
10	20	10	2				2		
11	20	10	2					2	
12	20	10		2	1				
13	20	10		2		3.25			
14	20	10		2			2		
15	20	10		2				2	
16	20	10			1	3.25			
17	20	10			1		2		
18	20	10			1			2	
19	20	10				3.25	2		
20	20	10				3.25		2	
21	20	10					2	2	
22	10	10	4						10
23	10	10			2				10
24	10	10				6.5			10

All of these mordants leave some film on the slide when used. This seems necessary for one cannot stain flagella, it seems, when there is no film at all. The thickness of the film depends on the composition of the mordant, its age and the length of time it is allowed to act. Those containing the silver sol (Nos. 22-24) give the least film when fresh, but the others give less as they age until none is formed. "Renewed" mordants may give less film than others.

Soon after their preparation (fresh to one day old) some of these mordants may not require more than one and one-half to two minutes for mordanting, but when older (two to three days) they generally require at least ten minutes. Those containing $AlCl_3$ excepting No. 22, or $ZnCl_2$ do not work when fresh. Apparently all of these mordants contain considerable material in a colloidal state. After standing four or five days a heavy precipitate forms and they often fail to work; i.e., they no longer form a film on the slide. They are always heated when prepared but not for mordanting. They differ from many other mordants in containing less dye. Some dye is necessary but not much. Any one of them may be prepared in a few minutes as the only solution that needs to be fresh is the tannic

acid. Large numbers of stained preparations would have to be compared to determine their relative merits. For general use Nos. 1, 3, and 22 would doubtless prove sufficient and very satisfactory. However, it is interesting to know that all mordants in this list gave excellent results with the organisms used in this work.

STAINS: Glycerin as an Adjuvant to Bacterial Dyes, Huntoon, F. M. Am. J. Clin. Path. **1**: 317, 1931.

Huntoon presents formulae embodying glycerin which not only preserves the stains but gives a clearer background.

The formulae follow:

THE GRAM STAIN

Glycerin Crystal Violet Gram

The solutions are made up as follows:

Mix:

Fifteen parts, 3 per cent solution of crystal violet in 95 per cent alcohol.

Eight-five parts, 30 per cent solution of glycerin in water.

Counterstains:

A. Red counterstain. Mix 10 c.c. of carbolfuchsin with 100 c.c. of a 25 per cent solution of glycerin in water.

B. Brown-yellow counterstain. Shake up 2 grams of Bismark brown in 100 c.c. of water and filter. To the filtrate add 30 to 40 c.c. of glycerin and mix.

Glycerin Carbolfuchsin

Phenol, 5 per cent, 75 c.c.

Glycerin, 25 c.c.

Saturated solution of basic fuchsin in 95 per cent alcohol, 10 c.c.

This combination stains as well as the usual carbolfuchsin but does not tend to precipitate either in the bottle or on the slide even if boiled. It also gives clearer backgrounds.

Glycerin Methylene Blue

Twenty-five per cent solution of glycerin in water, 100 c.c.

One per cent solution of sodium hydrate, 1 c.c.

Saturated solution of methylene blue in 95 per cent alcohol, 10 c.c.

This mixture stains equally well or better than the usual Loeffler's methylene blue, contains one-third the amount of dyestuff, and gives sharper pictures.

An ideal blue counterstain for use with the carbolfuchsin tubercle bacillus stain is prepared by adding 2 c.c. of saturated alcoholic solution of methylene blue to 100 c.c. of a 25 per cent solution of glycerin in water.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Determinative Bacteriology**

THIS is Volume II of the completely revised and greatly extended manual which has long been a familiar companian of bacteriologists the world over.

The first section (172 pages) covers general bacteriology while the second section (759 pages) is devoted to special bacteriology in great detail.

There are three appendices concerning filterable viruses, the important pathogenic protozoa, and the bacterial flora found in certain habitats.

The changing concepts of bacteriology make a comprehensive review of the subject, such as these volumes furnish, of great value and utility and furnish to the bacteriologist and the clinical laboratory a reference work of unquestioned value.

Bioassays. A Handbook of Quantitative Pharmacology†

THIS volume represents the most complete and comprehensive summation of technic, methods, procedures, and experimental results which has yet appeared in the field of bioassays. Some five thousand five hundred specific references are quoted from the more than seventeen thousand which were consulted by Dr. Munch in the preparation of the manuscript.

The main body of the text is made up of nine chapters. The first of these deals with the nature and necessity of bioassays, the second with technic and the third with certain mathematical considerations bearing on the accuracy and interpretation of bioassays. The next four chapters deal with drugs affecting the nervous system, the circulation, the respiration, and the muscles. Chapter eight discusses the bioassay of glandular products including the adrenals, liver, pancreas, parathyroids, pituitary gland (both the anterior and the posterior lobe), sex glands (including the male and female sex hormone) and the thyroid. The last chapter takes up the bioassay of special types of drugs including anthelmintics, antipyretics, antisyphilitics, arsenic, biological products, cathartics, diaphoretics and sialogogues, diuretics, emetics, insecticides, mydriatics and miotics and finally the vitamins ranging from A to F and including PP (vitamin G).

With the subject matter indicated above the more than nine hundred pages of the volume deal in a most exhaustive manner. Practically nothing of importance has been omitted, and Dr. Munch has made the reviews of methods and results as published by many workers sufficiently complete and detailed to enable others to use these methods and obtain

*Determinative Bacteriology. By Prof. Dr. K. B. Lehmann and Prof. Dr. R. O. Neumann. Authorized English Translation of the 7th revised edition edited by Robert S. Breed. Cloth. 868 pages, 42 figures. Alfred Hafner, New York.

†Bioassays—A Handbook of Quantitative Pharmacology. By James C. Munch, Director of Pharmacological Research, Sharp and Dohme, Pharmacologist, Bureau of Biological Survey, U. S. Department of Agriculture. x + 958, pages, 22 illustrations including 6 color plates. Cloth. 6 x9. The Williams and Wilkins Company, Baltimore, U. S.A.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

comparable results. This has been a task of heroic proportions and the author has completed it with a courage and a thoroughness which is seldom met in a field so new, so uncertain and so variable as is the subject of bioassay or quantitative pharmacology.

From a commercial and a pharmaceutical standpoint bioassays can only be considered as nuisances and makeshifts. They are expensive, time consuming and require much skill and experience on the part of those who carry them out. But from a clinical and practical standpoint they are imperative until sufficiently accurate and reliable chemical methods of assay shall have been developed for testing a considerable number of substances whose composition and therapeutic efficiency cannot yet be determined from what is known of their chemistry. And new compounds must of necessity be first tested on animals before they can be utilized intelligently and safely in the clinic. All of these phases of the subject have been admirably and comprehensively treated by Dr. Munch in his *Bioassays*. This book will be found indispensable by all those whose work deals with the physiological standardization of drugs. And it contains a vast amount of miscellaneous information, including much of the author's own experimental work and conclusions, which will be of great value to many workers in biological fields.

---

*Spezifische Veranderungen Des Blutserums**

IN THIS monograph the author presents results obtained in a careful and very painstaking study of physical properties of blood serum in various conditions, more especially carcinoma and tuberculosis. The method most thoroughly employed consists in determining the point where flocculation occurs on the addition of varying quantities of acetic acid and sodium vanadate.

Bendien starts out with the assumption that the various agents responsible for diseases bring about the appearance of qualitatively different proteins which can be recognized by means of his flocculation reactions. He states that sera of tuberculous and cancerous patients differ from normal sera with respect to the P_H at which flocculation occurs, and that his reaction can supply information as to early diagnosis and prognosis.

Observations are recorded also on the chemical examination of the blood in cases of eclampsia and epilepsy. Considerable deviations from normal reactions were found in cases of diabetes and nephritis.

Another part of his investigation deals with spectrographic examination of normal and pathologic sera. The book closes with a supplementary chapter devoted to the technic of spectrography.

—PHILIP LEVINE.

---

*Spezifische Veranderungen des Blutserums. Ein Beitrag zur serologischen Diagnose von Krebs und Tuberkulose. By S. G. T. Bendien, M.D., Sociological Laboratory, Flist, Holland; pp. 99; Jena, Germany; Gustav Fischer, 1931.

The Journal of Laboratory and Clinical Medicine

VOL. XVI ST. LOUIS, MO., SEPTEMBER, 1931 NO. 12

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. — CINCINNATI
PAUL G. WOOLLEY, M.D. — LOS ANGELES
J. J. R. MACLEOD, M.B. — ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. — ROCHESTER, MINN.
GERALD B. WEBB, M.D. — COLORADO SPRINGS
VICTOR C. MYERS, PH.D. — CLEVELAND
RUSSELL L. HADEN, M.D. — CLEVELAND, OHIO
JOHN A. KOLMER, M.D. — PHILADELPHIA
ROBERT A. KILDUFFE, M.D. — ATLANTIC CITY, N. J.
GEORGE HERRMANN, M.D. — NEW ORLEANS
T. B. MAGATH, M.D. — ROCHESTER, MINN.
DEAN LEWIS, M.D. — BALTIMORE
M. H. SOULE, SC.D. — ANN ARBOR, MICH.

Contents of this Journal Copyright, 1931, by The C. V. Mosby Company—All Rights Reserved

Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIAL

The Prognosis of Cancer

WITHIN recent years various methods, mainly based upon histologic minutiae, have been proposed as enabling or assisting in the determination of the prognosis of a malignant growth.

While it is not only possible but, in greater or less measure it has been demonstrated, that information of distinct prognostic value may be thus obtained, the problem has this much in common with the prognostic value in any disease, namely, that the prognosis of disease depends not only upon the disease but upon the patient who has the disease, and that it is directly influenced by the individual reactions and interreactions in each particular case. In other words, prognosis in disease may not with safety be based upon a single factor but represents rather a sum total of numerous and various factors which possess varied importance in the individual case.

MacCarty* has recently presented these factors in a succinct and admirable summary which, because of the importance of the subject, well deserves wide circulation.

*MacCarty, M. C.: Principles of Prognosis in Cancer, J. A. M. A. 96: 30, 1931.

The basic, underlying, and vital principle upon which MacCarty's position is based is that "it is a mistake, with present knowledge, to use any one criterion as the basis of clinical prognosis in cancer."

This is not to cast discredit upon histologic evidence leading to inferential deductions as to the potentialities of a given growth, nor to suggest that such studies be abandoned, but rather to emphasize the necessity for their evaluation and interpretation in the light of all the other factors concerned. Of these MacCarty considers there are fifteen which, in the order of their clinical importance, are as follows:

1. The presence or absence of glandular involvement and distant metastasis.
2. Fixation of growth.
3. Location.
4 & 5. Renal and cardiac efficiency.
6. Anemia.
7. Size of growth.
8. Age.
9. Direction of growth.
10. Loss of weight.
11, 12, 13, and 14. Cellular differentiation, lymphocytic infiltration, fibrosis, and hyalinization.
15. Duration of disease.

It will be seen that, while all the factors concerned with the strictly pathologic features of malignant neoplasms, that is, their histologic character as suggesting rapidity and character of growth and so on, are given their just due, the many other factors of equal and sometimes of even greater prognostic importance are equally emphasized.

In other words, MacCarty has again restated the old but ever-true dictum that not only the disease itself must be studied and weighed in the balance, but also the patient who has the disease, for in the last analysis it is the patient and his reaction, as well as his individual capacity to react, which determines in no small measure the ultimate outcome.

To the unbiased, the careful, and the competent observer, it is apparent that, in the present state of knowledge, all studies, whether clinical or laboratory, of cancer must be regarded as purely investigative and resulting so far only in the accumulation of data later to be accurately and finally evaluated.

So far, there is no criterion by which the ultimate outcome of malignancy in a given case can be definitely, finally, or infallibly foreseen.

—*R. A. K.*

Correction

In the August issue the illustrations for the second paper by Dr. Pollack were printed in the first paper through error. The entire paper with the correct illustration is reprinted in this issue, page 1194.

SEP 28 1931

INDEX NUMBER

Vol. XVI SEPTEMBER, 1931 No. 12

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., EDITOR
808 Professional Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Pathology
PAUL G. WOOLLEY, M.D.
Pasadena

Physiology
J. J. R. MACLEOD, M.B.
Physiological Laboratory, Marischal College, Aberdeen, Scotland

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology
W. C. MacCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry
VICTOR C. MYERS, Ph.D.
Western Reserve University, Cleveland

Experimental Medicine
RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

Immunology
JOHN A. KOLMER, M.D.
University of Pennsylvania, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Internal Medicine
GEORGE HERRMANN, M.D.
University of Texas, Galveston

Bacteriology
M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

Surgery
DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523-25 PINE BLVD., ST. LOUIS.

Entered at the Post Office at St. Louis, Mo., as Second Class Matter.

DR. GEORGE E. DEELY, Brooklyn, New York, died June 2, 1931, of knife wounds inflicted by a discharged employee who was caught in the act of burglarizing the house. Dr. Deely had been a member of the P. C. A. for nineteen years, at a cost of $247.00, but a previous claim had been paid him amounting to $176.80 leaving the net cost $70.20 for the $5,000.00 paid to his beneficiaries, the same day proofs of claim were received.

$4,618,969.44

has been paid to Physicians and Dentists for Sick and Accident Claims to July 1, 1931

Nearly thirty years continuous operation under same management. Purely mutual non-profit organizations. Conducted under supervision of Nebraska State Insurance Department where a deposit of $200,000.00 is maintained for protection of all members.

Cost: [**$13.00 yearly for each $5,000—$25 accident policy**
$17.00 yearly for each $25.00 weekly health policy.
Payable quarterly if desired.
One, two, or three policies in each Association may be issued to one person.]

PHYSICIANS HEALTH ASSOCIATION—PHYSICIANS CASUALTY ASSOCIATION
OMAHA *E. E. Elliott, Secy-Treas.* NEBRASKA

BRING YOUR GLASS PROBLEMS TO

HARRIS COMER

STEELE GLASS COMPANY

145-147 NORTH FOURTH STREET

GLASS APPARATUS GLASS BLOWING

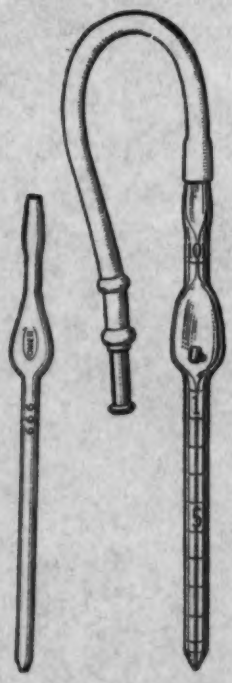
FOR CHEMICAL, RESEARCH, BACTERIOLOGICAL LABORATORIES

We offer the service of skilled workmen and our experience of 40 years in adapting glass to a multitude of scientific and industrial purposes

PHONE LOMBARD 5856

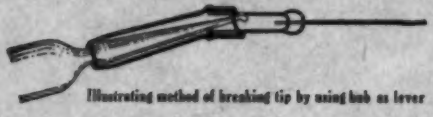
GLASS TUBE GLASS ROD

TRADE COMER MARK



comer

This trade-mark on the back of our Blood Count Pipette means that we guarantee a Greater Accuracy than is required by the U. S. Bureau of Standards.



Illustrating method of breaking tip by using hub as lever

Fig. 1

Patented

Comer's Window Needle *makes it possible to SEE that*

- The breakable tip of ampoule is not prematurely broken.
- The Blood shows in Glass Hub when needle is properly introduced into vein.

BLOOD CHEMISTRY KEIDELS

with Sodium Fluoride as used by the Pennsylvania Department of Health under the direction of Dr. John L. Laird

HARRIS COMER — PHILADELPHIA, PA.

A Marvelous Work—Now Ready

Histopathology of Skin Diseases

By Lee McCarthy, M.D.

Membré Correspondant Estranger de la Societe Francaise de Dermatologie et de Syphiligraphie; Dermatologist to the Garfield Memorial Hospital; Attending Dermatologist to the Providence Hospital, to the Gallingher Municipal Hospital; Associate Dermatologist to Children's Hospital; Consulting Dermatologist to the U. S. Naval Hospital, Washington, D. C., to the Florence Crittenden Home, Washington, D. C.; Member of the Baltimore and Washington Dermatological Society.

513 pages, 8½ x 11, with 175 half-tone illustrations and 54 plates in colors prepared by special artists. Price, special binding, $25.00.

Authoritative and Up-to-Date

In this new book the author presents the histological picture of the various skin diseases in agreement with the consensus of opinion of the different individuals who have studied them in detail. He gives the reader in an abbreviated form the anatomical changes in skin diseases as accepted by the dermatologists throughout the world and has added his personal views only in exceptional instances. A short clinical outline has been prepared in order to make the histological description more comprehensible.

54 Plates in Colors

One of the outstanding features of this book is the superb series of illustrations. The color plates are from drawings by two of the leading artists of Europe, and form an exact reproduction of the changes seen in slides. This exactness was made possible largely by the knowledge of cytology possessed by Mons. A. Bessin of Paris, one of the artists. The microphotographs are by Fraulein Bruny working in the Prof. Christeller's laboratory at the Virchow Krankenhaus.

The Edition is Limited—Send for Your Copy Today

The publishers feel that this work by McCarthy represents one of the outstanding contributions to the study of skin diseases made during recent years. No expense has been spared in the production of the illustrations and in the preparation of the text. It is a work that will live. The price is small when you consider the thousands of dollars its publication cost.

The C. V. Mosby Company, Publishers, 3523 Pine Boulevard, St. Louis.

You may send me a copy of McCarthy—HISTOPATHOLOGY OF SKIN DISEASES. Price, $25.00. ☐ I agree to pay in 30 days. ☐ Charge to my account. ☐ I agree to pay $5.00 on delivery and $5.00 each month for 4 months.

Name _____ Address _____

(JLCM)

Freas Electric Water Bath

17483

The Freas Electric Water Bath is intended for the treatment of materials which permit immersions, for example in the case of Wassermann reactions, inactivations, serological work, inspissations, paraffine embedding, and similar operations. These baths also are particularly suited for use with the penetrometer in asphalt testing for penetration, see A.S.T.M. Test D 5-25. They are intended also for fractional evaporation of alcohols, ethers and similar substances, and are widely used in the fertilizer industry and similar fields.

The baths consist of a heavy tinned copper tank, 1 in. of cork insulation, and outer shell of lead-coated ingot iron. The temperature is regulated within ± 0.2° C. by a differential linear expansion type of regulator. The baths have a constant level and a pilot light to show making and breaking of thermo regulator. Outer walls are finished in gray lacquer and have nickeled fittings. Temperature range up to 100° C.

17483. **FREAS ELECTRIC WATER BATH**—Inside Dimensions 12 × 12 × 8½ in. deep ---------- $125.00

17484. **Ditto**, but with inside dimensions 18 × 12 × 8½ in. deep ---------- 150.00

17485. **Ditto**, but with inside dimensions 36 × 18 × 12 in. deep ---------- 200.00

17483a. **TEST TUBE SUPPORTS**—For Freas Electric Water Bath; made of copper

For Tubes	18	20
Size, in.	¾ × 6	½ × 4
Each	2.75	1.50

Write for Bulletin No. 470

EIMER & AMEND

Est. 1851 Inc. 1897

Headquarters for Laboratory Apparatus and Chemical Reagents

Third Ave., 18th to 19th St. NEW YORK, N. Y.